PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/47, 16/18, C12Q 1/68, A61K 38/17 — A2

(11) International Publication Number: **WO 99/51740**

(43) International Publication Date: 14 October 1999 (14.10.99)

(21) International Application Number: PCT/EP99/02295

(22) International Filing Date: 2 April 1999 (02.04.99)

(30) Priority Data:
98400823.5 6 April 1998 (06.04.98) EP

(71) Applicants *(for all designated States except US)*: JANSSEN PHARMACEUTICA N.V. [BE/BE]; Turnhoutseweg 30, B–2340 Beerse (BE). UNIVERSITY OF WASHINGTON [US/US]; Seattle, WA 98105–4631 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MARTIN, Geneviève [FR/FR]; 27, rue du Lieutenant Princeteau, F–59800 Lille (FR). NEMOTO, Masami [JP/JP]; Dept. of Internal Medicine (III), Jikei University School of medicine, 1–17–1, Nishishinbashi, Minato–ku, Tokyo 105 (JP). DEEB, Samir, Sami [US/US]; 846 North East 104th Street, Seattle, WA 98125 (US). AUWERX, Johan [BE/FR]; 60, route d'Hasnon, F–59173 Millonfosse (FR).

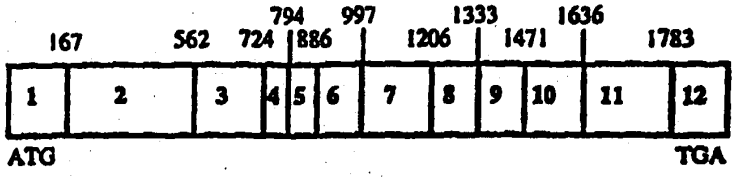
(74) Agents: ERNEST GUTMANN–YVES PLASSERAUD S.A. et al.; 3, rue Chauveau–Lagarde, F–75008 Paris (FR).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: NUCLEOTIDE SEQUENCE EXPRESSING HUMAN FATTY ACID TRANSPORT PROTEIN AND CORRESPONDING AMINOACID SEQUENCE. USE FOR THE REGULATION OF FATTY ACIDS METABOLISM



(57) Abstract

The invention relates to a nucleotide sequence which comprises a sequence involved in the expression of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of the Figure.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Nucleotide sequence expressing human Fatty Acid Transport Protein and corresponding aminoacid sequence. Use for the regulation of fatty acids metabolism.

The invention relates to nucleotide sequences involved in the expression of the human Fatty Acid Transport Protein (hFATP) and relates to the Fatty Acid Transport protein.

Fatty acids, especially long-chain fatty acids, are the structural components of several classes of lipids; they represent an important energy source, particularly with respect to glucose, for various tissues or organs. Fatty acids furthermore participate in several cellular signaling processes.

Excessive intracellular accumulation of fatty acids due either to errors in metabolisms or to increased supply can have severe pathological consequences. Furthermore, the involvement of fatty acids in several diseases such as insulin resistance and coronary artery disease made the inventors focuss research efforts on the mechanisms which control the homeostasis of these lipid moieties.

Interestingly, fatty acids are ligands and modulators of transcription factors, which control their own metabolism. This emphasizes the fact that the various metabolic pathways are not only controlled by substrate supply, but also by the participation of substrates and metabolic intermediates in regulatory phenomena.

Intracellular Fatty Acid (FA) concentrations are in part determined by proteic regulators, in particular by regulating import/export system that is controlled by several proteins including Fatty Acid Transport Protein (FATP) and acyl-CoA synthetase (ACS).

Fatty Acid Transport Protein (FATP) was first isolated in the mouse in 1994 by a functional cloning approach (Schaffer et al, 1994).

The inventors have now isolated and characterized the human FATP coding sequence and the gene comprising said coding sequence. They have furthermore identified the chromosomal location of the FATP gene, in a region

implicated in several metabolic diseases, suggesting that FATP is part of an important group of synthenic genes. hFATP is expressed in several organs (heart, brain, liver) and tissues (adipose tissue, muscle) and is especially highly expressed in insulin-sensitive tissues. Regulatory studies suggest that its expression is under the control of several transcription factors including PPAR (Peroxysome Proliferator Activated Receptor) (Martin et al., 1997). Especially, Martin G. et al., 1997, have shown that the expression of Fatty Acid Transport Protein is regulated by PPARα and PPARγ activators, such as fibrates or antidiabetic thiazolidinedione. The inventors have now determined that the expression of the *hFATP* gene is also under the control of the Retinoïd X Receptor (RXR) known to transduce the effects of retinoic acid on gene expression and involved in the regulation of lipid and glucose metabolism.

Therefore, FATP is an important target for therapeutic agents used in the treatment of several pathological states resulting from anomalies in regulation pathways of expression, especially of transcription or translation of the gene coding for FATP, either directly or through transcription factors.

The invention thus relates to genetic means and especially to nucleotide sequences involved in the regulation pathway of the long-chain fatty acid metabolism and therefore makes available new compounds appropriate for the definition of therapeutic means useful for treating pathological disorders related to the metabolism of long-chain fatty acids.

An object of the invention is therefore a nucleotide sequence which comprises a sequence involved in the expression, of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of Figure 2 or Figure 5.

The nucleotide sequence of the invention can be any type of sequence including any DNA especially genomic DNA, synthetic DNA, RNA and especially mRNA, said sequences being sense or antisense sequences.

Therefore, the invention relates to these sequences when they are independently of the conditions used to obtain them, thus including sequences either extracted from a biological sample, or cloned or synthesized including by

enzymatic or chemical processes.

By the expression "a sequence involved in the expression", it is intended within the present invention a sequence coding for the Fatty Acid Transport protein (FATP) and especially human FATP, or a sequence involved in the regulation of the steps required to express the FATP gene, especially the *hFATP* gene, or both sequences operably linked. Said regulation sequences can be derived or are derivable from the native genomic sequence of the FATP gene, especially *hFATP* gene.

Regarding sequence coding for the FATP, especially hFATP, the invention pertains to a nucleotide sequence which is capable, when placed under the control of appropriate regulation elements, especially regulation nucleotide sequences, promoter, enhancer, transcription sites, to be transcribed and under appropriate conditions to be translated into an amino acid sequence. Said aminoacid sequence can then possibly be processed, depending on the expression system chosen.

Especially, the human Fatty Acid Transport Protein which is expressed is characterized by the amino acid sequence that it comprises. Depending from the cellular host chosen for its expression, the structure of the obtained hFATP can vary, especially as the result of a maturation process, or of environmental conditions.

According to a specific embodiment of the invention, the nucleotide sequence comprises a sequence encoding the human FATP, corresponding to or comprising the nucleotide sequence having nucleotides 1 to 2222 of one of the sequences of figure 1, figure 2 or figure 3.

According to a preferred embodiment of the invention, the nucleotide sequence encoding the human FATP at least comprises the open-reading frame (ORF) corresponding to the human FATP represented on figure 2 or figure 5. Alternatively it comprises any fragment of said ORF, coding for a polypeptide having the functional properties of the human FATP as far as the transport of the long-chain fatty acids is concerned. The coding sequence of the human FATP gene is described on figure 1, figure 2 or figure 3.

Such a nucleotide sequence can be placed under the control of the native regulation sequences, or part thereof, present in the gene and enabling the expression of hFATP or can be placed under the control of heterologous regulation sequences according to well-known procedures.

According to another embodiment, the invention relates to a nucleotide sequence hybridizing in stringent conditions, with a probe comprising 50 to 2000 bp, preferably 50 to 300 nucleotides especially around 200 bp, said probe including at least 6, preferably 9 continuous nucleotides from the following sequence : CGGGGAGACGGGACGTGAAGGG.

As an example, a sequence which is advantageously used to provide for a probe appropriate to selectively hybridize with the nucleotide sequence of the invention comprises at least some nucleotides contained in the 5' non-coding sequence upstream from the ATG codon of the coding sequence of the gene expressing the hFATP.

A probe replying to this definition is considered to be specific for the human gene encoding the hFATP or derived genomic nucleotide sequences, expressing the human FATP. Thus, it does not hybridize with murine, rat or yeast cDNA sequences. Such a probe can be an amplification product resulting from amplification with primers wherein one primer is specific for the human hFATP gene, said primer comprising at least 6, preferably 9 nucleotides selected from the above nucleotide fragment.

The second primer can be taken in any region of the gene and especially in the sequence comprising nucleotides 1 to 2222 of the sequence of figure 1, or figure 3.

The stringent hybridization conditions used according to the invention can be defined with respect to the following parameters, referring to hybridization of a DNA probe, especially a cDNA probe with a total RNA or DNA:

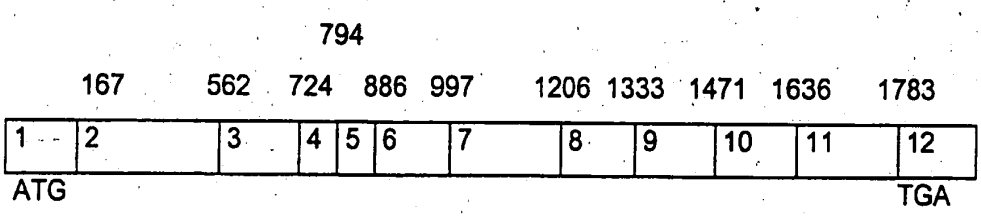
- hybridization at 42°C,
- 2 washing steps at 42°C for 10 minutes in 0.5 x SSC, followed by 2 washing steps at 65°C for 30 minutes in 0.5 x SSC.

Details regarding these conditions can be found in the examples.

According to a specific embodiment of the invention, the nucleotide sequence is a genomic DNA sequence and especially is the gene encoding the human FATP.

According to another embodiment of the invention, the nucleotide sequence comprises a transcription initiation site 61 bp upstream from the ATG codon. Especially, the nucleotide sequence comprises the sequence coding for the hFATP and the 5' region of the gene containing regulation sequences including the transcription initiation site.

The structure of the sequence of the gene between the ATG and TGA codons, can be represented as follows:



The respective size of each exons illustrated on figure 8 starting from position 1 at the ATG codon is the following:

exon 1 (167 pb), exon 2 (395 pb), exon 3 (162 pb), exon 4 (70 pb), exon 5 (92 pb), exon 6 (111 pb), exon 7 (209 pb), exon 8 (127pb), exon 9 (138 pb), exon 10 (165 pb), exon 11 (147 pb), exon 12 (158 pb).

The introns present between said exons, have respectively the following 5' and 3' ends: intron 2 (5' gtgaggcc...gaccacag 3'); intron 3 (5' gtgagtca...tcctgcag 3'); intron 4 (5' gtgagggg... cccctgcag 3'); intron 5 (5' gtactacg...ctctgcag 3'), intron 6 (...cgtcccccac 3'); intron 7 (5' gtgcacacc...cattccag 3'); intron 8 (5' gtgagcag...ctccctag 3'); intron 9 (5' gtgcgcag...tctgccag 3'); intron 10 (5' gtcaagct...gcctccag 3'); intron 11 (5' gtgcgagt...cactatag 3'). Intronic regions of a *hFATP* gene according to the invention are represented on figure 4 which discloses the genomic sequence encoding the human FATP.

Advantageously, the nucleotide sequence according to the above-given definitions comprises further sequences involved in the regulation of the

expression of the gene coding for the human FATP, present in the non coding 5' and/or 3' regions of the gene.

The sequences involved in the regulation of the expression of the gene include sequences containing the promoter region, the transcription initiation site and other regions involved in the activation of the expression or in enhancement thereof.

Regulation of the expression also involves the regulation of the translation of the gene and the corresponding sequences.

Variants of the above-defined nucleotide sequences comprise a sequence which either specifically hybridizes in high strigency conditions with primers having the following sequences primer 1 AAGGTCAATGAGGACACAATGG (sense), primer 2 CGAGTAGGTAGTGATCGTGCAG (antisense), or is the amplification product obtained with the following sequences, or hybridizes in high stringency conditions with said amplification product.

Another variant of the sequence is a genomic sequence coding for hFATP and which hybridizes in high stringency conditions with a probe containing around 200 pb, said genomic nucleotide sequence preferably comprising a sequence involved in the regulation of the gene coding for the hFATP.

A specific probe containing around 200 bp which is capable of specifically hybridizing with a genomic sequence coding for FATP can for example be found in the sequence of exon 2.

Another nucleotide sequence according to the invention is the mRNA which is a sequence obtainable by transcription of a genomic nucleotide sequence coding for the hFATP and replying to the above definition.

The mRNA sequence obtainable from the gene coding for the hFATP comprises the nucleotide sequence complementary to the sequence of figure 1, or figure 3, having 2 kb. Preferably, it is a sequence including a nucleotide sequence of 4.4 kb capable of hybridizing in high stringency conditions with the sequence of figure 1, figure 2 or figure 3.

The invention also specifically relates to the cDNA as obtained by reverse transcription of the mRNA defined above. Such cDNA sequence comprises the sequence of figure 1 or figure 3 containing nucleotides 1 to 2222 or the sequence of figure 2.

The invention also concerns polynucleotides selected among the following sequences:

- AAGGTCAATGAGGACACAATGG,
- CGAGTAGGTAGTGATCGTGCAG,
- a sequence comprising or corresponding to sequences involved in the regulation of the expression of the gene encoding the hFATP,
- any fragment of a nucleotide sequence defined above especially derived from the sequences disclosed in figures 1, 3 or 4, for instance by deletion mutation or insertion provided the essential biological properties of the native sequences are maintained, specific for the *hFATP* gene, including fragments that can be used as primers for amplification reactions, or a probe for hybridization. The above cited biological properties are described in the examples which follow.

A further object of the invention is a vector, for the cloning or for the expression of a sequence defined above, said vector comprising, inserted in site not essential for its replication, a nucleotide sequence as defined above.

Any appropriate regulation sequence, including heterologous sequences with respect to the *hFATP* gene, and especially nay appropriate promoter, can be used for the expression of the nucleotide sequence of the invention cloned into the vector. The selection of these regulation sequences can depend upon the cell host which is used to carry out this expression.

Especially, the vector is a plasmid or a phage. Advantageously, it further comprises a reporter sequence such as the CAT gene or the sequence of the luciferase gene.

The sequence of the reporter gene which is present in the vector can be under the control of an heterologous regulator region especially under the control of the regulation sequences of the gene encoding the hFATP.

The invention also relates to recombinant cells especially procaryotic cells or eucaryotic cells and advantageously insect or mammal cells.

The invention is also directed to the human Fatty Acid Transport Protein comprising the amino acid sequence of figure 2, or figure 5 or to any polypeptide fragment having the properties of FATP regarding intracellular transport of fatty acids, or to fragments recognized by antibodies directed against the human FATP.

The hFATP has a calculated molecular weight of 71 kDa and an apparent molecular weight of 63 kDa in Northern Blot analysis.

According to a specific embodiment of the invention, the hFATP has an isoelectric point of 8.5 and replies to the amino acid sequence of figure 2 or figure 5. According to another embodiment, this sequence is encoded by a nucleotide sequence as defined above.

Advantageously, said human hFATP is processed and especially devoid of its signal peptide.

The hFATP of the invention can be either under its glycosylated form or can be devoid of its glycosylation groups.

The invention also relates to antibodies specifically directed against hFATP.

In view of the various properties which have been identified with respect to the FATP in the regulation pathway of the long-chain fatty acid, the invention provides means that can be used in therapeutic compositions especially for modulation of the intracellular level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

Alternatively, the composition of the invention can be used for the therapeutic modulation of the blood level of long-chain fatty acids.

The inventors have especially found out that the involvement of human FATP in the modulation of the metabolism of fatty acids, especially long-chain fatty acids, can provide a way to treat pathological states related to various diseases and for example to obesity, cardiomyopathies and diabetes, specially diabetes non-insulino dependent.

According to the invention, the expression "treatment" relates to the capacity of a composition or compound, to prevent the occurrence of a pathological state or to control such pathological state or to improve the condition of a patient suffering from a pathological state, where this pathological state is the result or involves an abnormal regulation of the long-chain fatty acids metabolism.

Especially, the invention relates to such a composition which comprises an inhibitor of the expression of the FATP gene, for instance for the treatment of obesity or related diseases.

Alternatively, the invention relates to a composition which is capable of enhancing the expression of the *hFATP* gene for the treatment of cardiomyopathies, diabetes or related diseases.

The invention further provides means enabling assaying the capacity of chosen compounds to have an agonist or antagonist activity with respect to the expression of FATP, especially human FATP, in various tissues. Such assay can hence comprise the steps of:

- contacting a culture of cells of a specific tissue, said cells containing the *hFATP* gene, with a tested compound;

- detecting the effect of said compound on the expression of the FATP gene.

The invention also relates to a method for the screening of the expression of the hFATP protein in determined cells when these cells have been contacted with a determined compound, which method comprises the step of detecting the transcription of the mRNA in the cells or cell extracts.

In a specific embodiment of the invention, the screening method comprises

a) measuring the level of transcription of the mRNA in cells or cell extracts, wherein the cells have previously been contacted with the determined compound, in conditions enabling the interaction of said cells and said determined compound;

b) measuring the level of transcription of the mRNA in the same cells

species as in step a) or on extracts of these cells, wher in these cells have not been previously contacted with the assayed compound;

c) comparing the level of transcription obtained in steps a) and b).

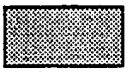
Preferably the cells which are used to carry out the above screening methods are selected among the group of live cells, heart cells, adipose tissue cells, skeletal muscle cells. The above defined methods can allow the identification of agonists or antagonists of the expression of the *hFATP* gene.

Specific elements of the invention are further disclosed in the examples and in the figures:

Figure 1: nucleotide sequence of clones containing 2 kb sequences obtained from a cDNA library of human adipose tissue. The sequence on the upper line corresponds to the sequence encoding the hFATP protein and the sequence on the bottom line corresponds to the alternative form of splicing found in the analyzed clones

Figure 2: Alignment of the nucleotide sequences of hFATP1, mFATP (MMU15976) and rFATP (RNU89529) and of the primary amino acid sequence, using the J. Hein method. The glycosylation sites are indicated by an asterisk and are boxed. The sequence of 11 amino acids common to the members of the family of AMP-binding proteins is boxed on the alignments.

□ Amino acid different from the murine sequence



Consensus sequence of 11 amino acids characterizing the family of AMP-binding proteins

□ Potential glycosylation site.

Figure 3: a variant of the nucleotide sequence of clones encoding the human FATP protein and obtained from a cDNA library of human adipose tissue.

Figure 4: sequence of the genomic sequence expressing the human FATP

protein, including the introns (lower case) and the exons (upper case).

Figure 5: The amino acid sequence encoded by the nucleotide sequence of figure 3 and corresponding to the human FATP protein.

Figure 6: Comparative analysis based on the Garnier-Robson, Chou-Fasman and Kyte-Doolittle algorithms of the murine and human FATP proteins.

Figure 7: Primer extension with the reverse GM6 primer on 10 µg of human visceral adipose tissue total RNA.

Figure 8: Provisional genomic structure of the hFATP gene. A. Genomic structure of hFATP with its 12 exons and 11 introns. The numbers in the boxes surrounding the exons indicate the exon numbers. The numbers above the boxes indicate the position of the introns. B. The approximate size of introns 2 to 11 (bp) is indicated between the dashes separating the two ends of the donor and of the recipient. Intron 6 was identified by sequencing the PAC clone. C. Alternative splicing leading to hFATP1 or its alternative splicing product hFATP. The relevant intron is intron 1, hFATP1 is normally spliced as in mice. The splicing of the variant of hFATP1 resumes beyond the intron in the coding sequence but changes the resading frame.

Figure 9. The 19p13 region. The arrow indicates the position of hFATP on chromosome 19, the shaded zone represents the frequency of the genes encountered in the different regions of the chromosome. The arm p of the chromposome is represented at the top. The region q is represented at the bottom.

Figure 10. A. Dose response of the effets of 9c-RA on FATP and ACS gene expression on FAO cells

Cells were kept 18 hr in serum-free medium and treated for 6 hr with increasing

concentrations of 9c-RA (10^{-8} M to 10^{-4} M). Control cells were incubated with BSA and vehicle. 30 µg of total RNA were loaded per lane. Northern blot analysis was carried out and blots were hybridized with FATP, ACS, and actin probes. Quantification was performed with an imaging densitometer (Biorad GS-670). Values were normalized by comparison with actin control probe and results are expressed as percent of control signal, relative arbitrary unit (R.A.U.).

Figure 10.B. Dose response of the effects of 9c-RA and at-RA on FATP gene expression on Hep-G2 cells.
Cells were kept 18 hr in serum-free medium and treated for 6 hr with (10^{-8} M to 10^{-4} M) either 9c-RA and at-RA. Controls were incubated with BSA and vehicle. Northern blot analysis was carried out and blots were hybridized with an FATP and actin probe.

Figure 11. A. Dose response of 9c RA on differentiated 3T3-L1 cells.
Differentiated 3T3-L1 cells were treated 24 hr with different concentrations 9c-RA (0-10^{-6}M). Northern blot analysis was carried out and blots were hybridized with an FATP and ACS probe. Actin was used as control probe. Δ means differentiated.

Figure 11.B. Comparative induction of FATP and ACS in FAO and Caco2 cells.
FAO(A) and CaCo2 (B) cells were serum deprived 18 hr and then treated during 6 hr with 9c-RA (10^{-6} M). Northern blot analysis was carried out as described and blots were hybridized with an FATP, ACS and actin probe.

Figure 12. Nuclear run on analysis of the effect of 9 cisRA on FAO and 3T3-L1 differentiated cells.
FAO cells were treated for 2 h with 9c-RA (10^{-6} M) after 18 h of growth in serum deprived conditions. Differentiated 3T3-L1 cells were treated during 12 h with the same concentration of 9c-RA. Transcription rates were then determined for

the FATP, ACS and GAPDH genes in from control cells (-RA) or 9c-RA treated cells (+RA). A Bluescript (BS) template was used as a control. Densitometric scanning of the results is depicted at the right panel.

Figures 13, 14 and 15

Dose-response of 9c-RA, at-RA and TTNPB and: in non-differentiated and differentiated 3T3-L1 cells.

Differentiated (3T3-L1 Δ) or non-differentiated (3T3-L1) cells were treated 24 hr with each of the retinoids indicated. Northern blot analysis was carried out and blots were hybridized with an FATP (figure 13), ACS (figure 14) and LPL (figure 15) probe. Values were normalized by comparison with actin control probe and results are expressed in percent of control (R.A.U.).

Figure 16. Oleate uptake assay on 3T3-L1 differentiated cells.

Differentiated cells 3T3-L1 (3T3-L1 Δ) were treated for 24 hr with different concentrations of TTNPB (10^{-9} M to 10^{-5} M) and ^{14}C oleate uptake studies were carried out. Results were normalized after protein quantification and expressed as % of control. Significant differences were indicated by an asterisk * $P<0,05$.

Figure 17. Tissue expression of FATP in different human tissues. The human probe of the ß-actin has enabled checking the regularity of the depots. The additional band obtained with this probe represents the actin specific of the muscle which is thus highly expressed in muscle and brain.

Figure 18. Expression of FATP in different human cell lines and comparison with the NCI adipose tissue: surrenal cortex carcinoma cells, THP1: monocytes, THP1 diff: THP1 differenciated with PMA, Caco2: cells of colon adenocarcinoma, HepG2: liver hepatoma cells, Hep3B: hepatocytes, JEG-3: chorion carcinoma cells.

Figure 19. Distribution of FATP and LPL in muscular skeletal tissue, adipose

tissue, liver, colon, and intestine tissues in human. Sc: sub-cutaneous, Vis: visceral.

Exemple 1

1. MATERIALS AND METHODS

1. Materials

BRL 49653 and fenofibric acid were provided by Janssen Research Foundation, in Beerse, in Belgium, by the Fournier laboratories, in Daix, in France and by Ligand Pharmaceuticals, in San Diego, in the United States. The retinoic acids *9-cis-retinoic acid* (9cRA), *all-trans-retinoic acid* (at-RA), were provided by Ligand Pharmaceuticals. All the other products, unless otherwise stated, were obtained from Sigma (St Louis, MO).

2. Human biopsies

The various omental and subcutaneous human adipose tissues were collected from normal or obese subjects during plastic or reconstructive operations. The muscular tissues were collected from patients who had undergone an operation in the hip region. They were the liver or skeleton muscle tissue. All the tissues were immediately frozen in liquid nitrogen.

3. Animals and treatments

Male rats of the Wistar strain were treated for various lengths of time with fenofibrate mixed with the feed in powdered form (weight/weight) or BRL 49653 by gavage, at the concentrations indicated. The weight of the animals and the intake of feed were recorded daily for the 7-day treatments and every two days for the 14-day treatments. The treatments with the fibrates did not cause significant changes in the quantity of fee consumed by the animals. Since each rat consumes approximately 20 grams of feed per day, the doses of 0.5, 0.05 and 0.005% (weight/weight) of fenofibrate correspond to 320, 32 and 3.2 mg/kg of body weight/day. At the end of the treatments, the rats were sacrificed by

exsanguination under ether anaesthetic. The triglyceride and cholesterol levels were measured using a colorimetric detection kit (Boehringer-Mannheim). The various tissues were collected, rinsed in 0.9% NaCl and immediately frozen in liquid nitrogen. The liver and the epididymal adipose tissue were weighed. The effects of starvation were studied on rats from which food had been withdrawn 14 hours before being sacrificed.

4. Cell culture

The cells of the different hepatic lines used were: Fa 32, a rat hepatoma cell line derived from Faza967 (Deschatrette et al., 1974), the mouse AML-12 hepatocytes (Wu et al., 1994), the human hepatoma line HepG2 and the FAO cells, a well differentiated subclone of the rat hepatoma line H4 II EC3. All the cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM), supplemented with 10% decomplementized foetal calf serum, except AML-12 and FAO. The AML-12 cells were maintained in DMEM/Ham's F-12 medium supplemented with insulin, transferrin and selenium (ITS, Collaborative Research), and dexamethasone (0.1 µM). The FAO cells were cultured in DMEM/Ham's F-12 supplemented with 10% decomplementized serum, penicillin (200 IU/ml) and streptomycin (50 mg/ml).

The mouse preadipocyte cell lines ob1771 (Negrel et al., 1978) and 3T3-L1 (ATCC) were maintainted in DMEM medium with 10% lipid-free and decomplementized foetal calf serum. The 3T3-L1 cells were differentiated by a two-day treatment with dexamethasone (0.1 µM), isobutylmethylxanthine (0.25 mM) and insulin (0.4 µM). These cells, following initiation of differentiation, were maintained for a further 8 days with insulin until their complete differentiation was obtained.

The L6 muscle cells were cultured in DMEM with 10% foetal calf serum up to confluence followed by spontaneous differentiation.

The Caco2 colon carcinoma cells (ATCC) were cultured in DMEM medium and 15% decomplementized foetal calf serum as well as antibiotics and non-essential amino acids. Above 80% confluence, the cells differentiate

spontaneously for 20 days.

5. RNA analysis

5.1. Extraction of RNA and quantitative analysis

The RNAs of the tissues and cells were prepared by the technique using caesium chloride and guanidine isothiocyanate (Chomeczynski et al., 1987). For the adipose tissue, a first step of centrifugation at 4°C of the homogenization product was added in order to remove the lipids, which are solidified at the surface, and to continue the extraction under the best conditions. The *dot-blot* and *Northern-blot* hybridizations of the total or messenger RNAs after separation on a poly A column (Stratagene) were carried out as described in Auwerx et al. (Auwerx et al., 1988). Before being deposited, the RNAs were assayed in duplicate by spectrophotometry (Pharmacia Ultrospec 2000), the difference tolerated between the values was less than 5%. The various messengers were measured using cDNA fragments obtained by *reverse transcription*, rt-PCR or by restriction, as probes. The probes were labelled with a *random primed labelling* kit (Boehringer-Mannheim) and α-^{32}P]dCTP (NEN, Boston, MA). The filters (nylon and nitrocellulose, Pall Filtron) were hybridized overnight at 42°C with 10^6 cpm/ml. The washes were carried out for 10 minutes at 42°C and for twice 30 minutes at 65°C in a 0.5 X SSC buffer and 0.1 % SDS. Next, these blots were exposed to autoradiographic films (X-Omat AR or Biomax MS, Kodak). The autoradiograms were analysed by densitometric *scanning* (Biorad GS 670 densitometer).

5.2 Measurement of the transcriptional activity by run on or transcription in vitro

The nuclei of the cells and of the tissues were prepared according to the Nevins technique (Nevins, 1987). The total RNAs labelled with α-^{32}P]UTP (3000 Ci/mmol) (NEN, Boston, MA), in quantity of equivalent radioactivity, were hybridized on membranes on which there had been deposited 5 µg of the different plasmids or 1 µg of the restriction fragments of the genes of interest.

5.3. Primer extension

The location of the site of initiation of hFATP was determined as described in Sambrook et al. (Sambrook et al., 1989). An antisens oligonucleotide, starting at +6 bases from the site of initiation of translation, was used 3'-CCC GCA TCC CTT CAC GTC CCG TCT CCC-5'. 10 µg of total RNA of human adipose tissue were precipitated with 500,000 cpm of primer labelled in 5' using T4 polynucleotide kinase (Amersham, Courtaboeuf, France with γ-^{32}P]ATP, denatured for 5 minutes at 95 °C and annealed for 90 minutes at 65°C in a hybridization solution. The extension was carried out at 42°C for one hour with 50 U AMV-RT (*Life Technologies, Paisley*, GB) and 100 U MMLV-RT (*Life Technologies*, Paisley, GB). A control sequence reaction was used as standard mass marker to locate the 5' end of the extension product.

6. DNA techniques

6.1. Screening of complementary DNA libraries and of genomic DNA of phages

Three libraries were used to isolate the FATP gene, including a commercial cDNA library (*Human fat cell 5'-stretch plus cDNA library* HL3016b, *oligod'1-primed, Clontech laboratories, Inc.* USA). After titration of the libraries, 10^6 *plaque forming units* (PFU) served for the infection of 6 ml of the respective competent bacteria (Y1090, XL-1 and SRB) OD_{600}=1, maintained in Luria Bertani medium with 0.2% of maltose and 10 mM $MgSO_4$. The adhesion of the phages to the bacterial walls was carried out for 15 minutes at 37°C in 14-ml polypropylene tubes. The mixture was then spread with TOP agar (*NZ amine, casein hydrolysate, yeast extract* (NZY) + 0.7% agarose) on 10 culture dishes 15 cm in diameter. The lysis was carried out overnight at 37°C. Two blottings on nitrocellulose filters (NEN, Boston, MA) were carried out on each dish, taking care to note the orientation of the dishes. The filters were then treated in a denaturing, neutralizing and washing solution, and then fixed under UV or with heat. The hybridization, washing and exposure conditions were the same as those used for the RNA techniques. In each screening, a double-stranded probe was used, derived from rt-PCR or from restriction. The probe was for

example a 652 pb fragment, amplified with the primer sense GM1 (ou 382) (5'-ATG CGG GCT CCT GGA GCA GGA ACA -3') and antisense GM2 (ou 399) (3'-CTG CGT GTC AGG CAG GAT GCT TCT AGG CCC-5') covering the 5' end of mFATP cDNA. The clones isolated from a first screening were then purified by two additional screenings. A final step consisted in amplifying the titre of the phage clone isolated.

6.2. Subcloning of the *hFATP* phage clones

The phage DNA was then prepared according to the Sambrook technique (Sambrook et al., 1989) or amplified by PCR with a *high fidelity polymerase* (Boehringer-Mannheim), and then mapped and excised at the level of the cloning site in the phage in order to then introduce it into a *bluescript* (BS) type vector, pBS-KS. The inserts could thus be amplified, sequenced, mapped and manipulated more easily.

6.3. Sequencing and alignment of sequences

The first clones were sequenced manually with the T7 sequencing kit (Pharmacia) or on a PCR product after treating the inserts with a phosphatase and an exonuclease I in order to remove the primers and the dinucleotides remaining whch interfere with the sequencing reaction. The product of sequencing was then deposited on a 6 or 7% acrylamide gel, the migration lasted 3-4 h at 60 mA. The sequence was read on the autoradiogram and could be as high as 200 bp.

The sequences were then prepared on an automatic sequencer (ABI 377, Perkin Elmer) with a PCR sequencing reaction using fluorescent terminators (ABI Prism big dye terminator, cycle sequencing ready reaction kit, Perkin Elmer Biosystem), the reading is automatic and may be as high as 700 bp. The sequence alignments and searches were carried out in a first instance with *Genbank* by means of the *Lasergene Navigator* software (DNA Star) and then in a second stage using the Internet on a national center for biotechnology information (NCBI) server. The search for homology for the cDNA fragments

was carried out using the *Blast search* programme. The *online mendelian inheritance in man (OMIM)* searches made it possible to identify the genes located on the same chromosome as hFATP.

6.4. Search for introns

The search for the intron/exon was carried out by PCR amplification on genomic DNA by comparison with amplifications on cDNA (phage clone, plasmid) or rt-PCR. Direct sequencing on plasmid artificial chromosome (PAC) (Genome systems Inc., St. Louis, USA) were undertaken. The junctions were identified after alignment of the sequences.

7. Measurement of the transport of oleate

7.1. Preparation of the fatty acids

The labeled fatty acids (^{14}C oleate, 40-60 mCi/mmol, NENE) are incubated in the presence of albumin in a ratio of between 0.5 and 2 for 45 minutes at 37°C in 1X Hank's solution.

7.2. Measurement of transport

The cells treated with the various activators are rinsed with a Hank's solution and then incubated for 1 h with serum-free and glucose-free medium. The cells were again rinsed with a 1X Hank's solution with 0.2% of *bovine serum albumin* (BSA) at 37°C and then at 23°C, the transport being measured at room temperature. The incubation of the cells with labeled oleate lasts for 1 minute, the stop solution was an ice-cold Hank's solution. The cells were lysed with 0.1% SDS, the proteins were assayed on 20µl, the remainder was supplemented with 4 ml of scintillation liquid and counted.

8. Chromosomal location

The FATP probe was labelled with digoxigenine-11-dUTP by nick translation (Boehringer-Mannheim). The probe was purified and concentrated by precipitation in order to obtain a concentration of 50 ng/µl. The chromosomal

preparation was obtained from T lymphocytes stimulated with phytohaemaglutinin. The cells thus stimulated were synchronized after 48 h of culture by the action of methotrexate for 17 h and then the cell cycle was unblocked by the addition of thymidine. After 4h 30 min of action, the cells were blocked in the metaphase by the action of colcmid. After a hypotonic shock and fixing, the preparation was deposited on a slide; to obtain good precision, the techniques of G bands obtained by the action of trypsin and after Giemsa staining (GTG banding) (Francke et al., 1978) and the FISH technique were combined. The metaphases were then selected on a DMRXA microscope (Leica). The were karyotyped and stored in a software for image analysis (CHANTAL) developed by Leica. After non-isotopic in situ hybridization (Geffroy et al., 1995) with the FATP probe and detection, the metaphases were examined with the aid of a fluorescence microscope (Leica type, DMRXA) combined with a Leica image analyzing system. The software allowed superposition of the metaphase in the G band and in FISH.

2. ISOLATION AND CHARACTERIZATION OF THE HUMAN *FATP* GENE

1. Results

1.1. Isolation of the complete cDNA from the human gene for *FATP*

1.1.1. Purification of the hFATP clones

Several human adipose tissue cDNA libraries, (including one commercial human fat cell 5' stretch plus cDNA library HL3046b, oligodT primed, Clontech library) were screened with a murine probe in order to isolate various cDNA clones of the human *FATP* gene. The first screening was carried out on the commercially made human adipose tissue cDNA library (Clontech). The probe used was a 5' fragment of 654 bp (position 1-654) amplified by rt-PCR on mouse adipose tissue RNA with primers 382 (5'-ATG CGG GCT CCT GGA GCA GGA ACA-3') and 399 (3'-CTG CGT GTC AGG CAG GAT GCT TCT AGG CCC-5') or alternatively (GTG TCA GGC AGG CAG GAT GCT CTC). This probe was inserted into a *bluescript* plasmid, pBS-KS, at the level of the EcoRV site and the sequence of this plasmid, mFATP399, revealed 100% homology

with the murine sequence. The screening provided 23 positive clones from 10^6 PFU, the probability of isolating a gene having a normal frequency being 1 out of 1 million. Next, a second *screening* was undertaken on another library under the same conditions for titration and spreading of the phages and for hybridization. This screening provided 6 clones which were identified as containing *FATP*. These clones were purified over three successive screenings.

The cDNA inserts, excised after digestion with EcoRI, provided fragments in a size interval from 300 bp to 2 kb. These clones were amplified by PCR using the lambda phage gt11 primers (λ gt11 *Forward* and λ gt 11 *Reverse)*. The sequencing of these PCR products allowed us to differentiate 1) the clones whose DNA sequences were homologous to *mFATP*; they are the partial *hFATP* clones, 2) the clones whose sequence was overlapping or even identical and 3) the clones whose DNA sequence did not align with *mFATP*. The latter category comprises false-positives which proved positive in hybridization because of homology with the probe used but whose sequence c differentiates them from *hFATP*. They may be genes already identified or *open reading frames* (ORF) not yet identified.

Three large inserts, homologous to *mFATP* (1.6 kb clone 2.a, 2 kb, clone 3.e and 1 kb, clone 3.g starting at nucleotide +84 following the ATG codon), were subcloned to a *Bluescript* vector at the level of the EcoRI site and completely sequenced over the sense and antisense part. These fragments were then aligned with the mouse sequence which was, with the rat sequence *rFATP*, highly homologous, the only two sequences known from 1994 to the end of 1997. The 1.6 kb clone aligned from position 487 on mice up to position 1941 which corresponds to the TGA codon, that is to say the end of the murine coding part. This clone therefore had an additional 3' part (147 bp) which was identified by rt-PCR as the 3'*untranslated transcriptional region* (3'UTR) and of the *FATP* gene, a region which is transcribed but not translated.

The 2 kb clones appeared to be identical during total amplification of these 2 kb with the λgt11F and λgt11R primers by PCR. The sequencing of these clones made it possible to differentiate them: *hFATP1* and an alternative

form of splicing. The open reading frame of this clone is 1941 bp for *hFATP1*, that is to say identical to the murine coding sequence of 1941 bp. Its alternative form of splicing had an 82-base deletion compared with *hFATP1*. This deletion is situated from position 168 to 249 on the mouse sequence or the *hFATP1* sequence. This deletion could result from an alternative splicing, which was thus analyzed with the genomic structure. These two clones had 22 bases of 5'UTR and 265 bases of 3'UTR.

1.1.2. Characteristics of the clones

A search in the NCBI library revealed 84% similarity at the nucleotide level between *hFATP1* and *MMU15976* (murine *FATP* sequence identification name) and 83% between *hFATP1* and *RNU89529* (rat *FATP* sequence identification name). The murine *FATP* sequence exhibits 93% at the nucleotide level with the *rFATP* sequence. A search for sequence homology with the entire library of genes listed by NCBI was carried out with the Blast search program. This analysis revealed 57% homology at the nucleotide level with the cDNA for rat acyl coenzyme A synthetase (D85100) with an alignment over 351 bases, 58% with the murine homologue of ACS (AF33031) with an alignment over 348 bases. The *hFATP1* sequence also exhibited 94% identity over 18 bases (D88308) and 56% over 210 bases with the human homologue of ACS. The center of this region of homology is indicated in Figure 2.

At the amino acid level, the protein exhibits 89% homology with the murine protein, mFATP, and 89% with the rat protein rFATP. rFATP and mFATP have 93% homology. Yeast Fat 1P had, for its part, only 54% homology with mFATP.

The hFATP protein comprises 646 amino acids, its calculated molecular weight is 71 kDa and its isoelectric point is 8.5. We compared the amino acid sequence of *hFATP1* and from its alternative splicing form. Said alternative from encodes a protein of 57 amino acids and ends with an ambre mutation which stops translation. By observing the translation in the three phases of the complete sequence, we noticed that the sequence following this termination

site aligned farther away in another reading frame with *hFATP1*. Such a phenomenon can only be due to a splicing error which can be confirmed by analyzing the genomic structure.

Analysis of the composition of the protein by the DNA Star software shows 40% of hydrophobic amino acids, which is in agreement with the fact that FATP is a transmembrane protein. hFATP has a signal sequence of 30 amino acids (von Heijne, 1986). The amino acid sequence of hFATP shows three potential glycosylation sites (N-X-S/N-X-T), these sites are conserved in mice, rats and humans. Only one of these sites is identical to the yeast Fat 1P protein whose amino acid sequence was published in 1997 (Faergeman et al., 1997). In a very advantageous manner, a sequence of eleven amino acids is perfectly identical and conserved between hFATP, mFATP, rFATP, Fat 1 and rACS. This oligopeptide (IYTSGTTGLPK) is common to members of the family of AMP-binding proteins. On the basis of this sequence conservation, the common evolutionary line for the FATP, Fat 1P and ACS genes can be confirmed.

The structural units characterizing the secondary structure of the FATP protein was studied by comparing its protein analysis using the protean program of the DNA Star software (Figure 6).

Using the kyte and Doolittle algorithms, the hydrophilicity profiles of hFATP and mFATP was compared (kyte et al., 1982). An analysis of the primary amino acid sequence based on the Chou-Fasman calculations was then carried out in order to locate the α and β sheets, and the bent and helical regions (Chou et al., 1974). This study was complete with a Garnier-Robson analysis locating the same regions but with a different method (Garnier et al., 1978). These analyses demonstrated that these proteins exhibited comparable profiles.

The 3'UTR region does not contain putative polyadenylation sites or destabilizing consensus sequence (ATTTA), which suggests that the complete message is much longer than 2 kb. This hypothesis was confirmed further to a commercial Northern-blot hybridization with a human *FATP* probe. The size of

the messenger is approximately 4.4 kb.

1.1.3. Identification of the site of initiation of transcription

In order to identify the site of initiation of transcription, a 5' extension, *primer extension*, with an antisense primer (reverse GM6) positioned in 5' of the coding region was carried out. The number of bases between the primer and the extension product was 34 bases, the primer was 27 bases long, which locates the site of initiation 61 bases upstream of the ATG. This distance is relatively short compared with the average distances of the sites of initiation which, in addition, may be highly variable. The location was checked with a *rapid amplification of cDNA ends* (RACE) technique.

1.2. Isolation of the human FATP gene and genomic characterization

1.2.1. Isolation of the human genomic DNA clone

In the second instance, a human genomic DNA phage library was hybridized with a human probe and 6 genomic clones were isolated from 10^6 PFU. The genomic characterization of human *FATP* was carried out by CPR on the genomic clones and total genomic DNA.

A PAC clone, derived from a plasmid capable of inserting fragments up to 120 kb was used. This clone was obtained using two primers, the first located from nucleotide 1267 to 1288 (GM3) and the second from nucleotide 1495 to 174 or more preferably from nucleotide 1469 to 1489 (GM4) in the sequence. The *screening* was carried out by PCR on human genomic DNA. The determination of the introns on this PAC was carried out by automatic sequencing using a sequencing technique developed for long DNA fragments (Fajas et al., 1997). Unlike the genomic clones previously isolated, the PAC14957 clone did not contain the pseudogene. Indeed, the primers used were intended to amplify the region which contained an intron, which made it possible to avoid again isolating the pseudogene. For the remainder of the characterization, this PAC14957 clone and the human total genomic DNA being checked were used.

1.2.2. Structure of the gene

On this PAC clone, the introns situated at the 3' end of the FATP gene up to position 997 were identified. Beyond this region, towards the 5' end, no alignment could be achieved on more than 3000 bp sequenced on a PAC 14957 clone by the technique of DNA walking towards the 5' region. Furthermore, this PAC clone did not appear to hybridize with oligonucleotides having the 5' part of the cDNA. In order to determine the positions of the other introns, a PCR strategy was applied to human genomic DNA in comparison with the rt-PCRs carried out on the human adipose tissue RNA (Figure 8). In order, the size of the exons is: 167, 395, 162, 70, 92, 111, 209, 127, 138, 165, 147 and 158 bp.

1.3. Chromosomal location of *FATP*

With the aim of determining the chromosomal location of *hFATP*, an *in situ* hybridization technique was used. Twenty interpretable metaphases, following the chromosomal preparation of T lymphocytes, were selected for hybridization with the *hFATP* probe. The *in situ* hybridization with the PAC14957 probe, a genomic clone of *hFATP*, labelled with digoxigenin, was located on chromosome 19, more precisely 19p13.1.

This region is very rich and 126 genes or locus responsible for disease were located in this region. Among the genes of greatest interest, at the level of the lipid and carbohydrate metabolism, situated in the same 19p13 region, there are: the familial hypercholesterolaemic locus, the insulin receptor, the atherogenic lipoprotein phenotype, apolipoprotein C-I and the locus involved in apo C-II deficiency.

2. Discussion

The human homologue of *mFATP* was isolated by screening a human adipose tissue cDNA library. Two messengers were differentiated: *hFATP1* which encodes a protein of 646 amino acids and *hFATP2*, the truncated form of

hFATP1, which encodes a protein of 57 amino acids. The functionality of hFATP2 is very unlikely and is derived from an alternative splicing. *hFATP1* comprises 12 exons and 11 introns.

The hFATP1 protein is hydrophobic in nature and its protein profile is very similar to that of mFATP. hFATP1 is a hydrophobic, transmembrane protein with three glycosylation sites and a potential signal sequence of 30 amino acids. Analysis of sequence homologies with other proteins made it possible to reveal 11 amino acids which are perfectly identical between ACS and FATP. These amino acids would constitute a binding site common to these two proteins. Shaffer and Lodish had already suggested that FATP and ACS could act in concert; the inventors have now shown analysis of gene regulation that *FATP* and *ACS* are regulated in a coordinated manner in tissues sensitive to insulin. FATP is thought to allow the entry of fatty acids into the cell and ACS is thought to convert them to active metabolic compounds, the acyl CoAs, for storing energy as a reserve or for the production of energy.

The location of *FATP* gene on chromosome 19, more precisely 19p13.1, is of interest since this region comprises numerous genes involved in the metabolism of fatty acids: the locus for apo C-II deficiency, the metabolism of glucose, the insulin receptor and the metabolism of cholesterol with the locus for familial hypercholesterolaemia. *FATP* is thought to form part of a syntenic group with one of these other genes and to segregate in the same manner. Thus, ap C-II deficiency causes a decrease in the LPL activity and is accompanied by accumulation of particles high in triglycerides. The insulin receptor has been very widely studied in order to search for the causes of insulin-dependent diabetes, an abnormality in this receptor prevents the action of endogenous and exogenous insulin. Familial hypercholesterolaemia due to a mutation in the receptor for the LDL particles causes the development of atherosclerosis and of cardiovascular diseases. All these manifestations have in common an accumulation of lipids in the bloodstream which may lead to insulin resistance.

Example 2

INDUCTION OF THE FATP GENE BY DIMER SELECTIVE RETINOIDS SUGGESTS THAT PPAR-RXR HETERODIMER IS ITS MOLECULAR TARGET.

Free fatty acids can be released from adipocytes by the hormone-sensitive lipase (HSL) or from tripglycerides-rich lipoproteins by lipoprotein lipase (LPL). Circulating fatty acids can then cross the plasma membrane either by virtue of their lipid solubility (Higgins, 1994) or be taken up by cells in a process mediated by the fatty acid transport protein (FATP). A second protein found to be involved in the process of long-chain fatty acid uptake is the Acyl Coenzyme A synthetase (ACS). FATP acts as a transporter of fatty acids, whereas the role of ACS is rather confined in preventing the efflux of fatty acids through an esterification process.

FATP and ACS mRNA levels are regulated in a tissue-specific manner by peroxysome proliferator-activated receptors (PPARs) (Martin et al., 1997). PPARs are members of the nuclear receptor gene superfamily. Expression of LPL, FATP and ACS is known to be under the control of hypolipidemic and hypoglycemic drugs, an effect mediated by PPARs. Three retinoic acid receptors, termed RARα, -β and -γ, and three retinoid X receptor, designated RXRα, -β and -γ, are classically thought to transduce the effects of retinoic acid (RA) on gene expression. Both 9-*cis* RA (9c-RA) and *all trans*-RA (at-RA) can directly bind and activate RARs, whereas RXR doesn't bind at-RA, but binds 9c-RA. RXR-PPAR heterodimers respond to both RXR and PPAR ligands (Kliewer et al., 1992).

RXR agonists function as insulin sensitizers and have beneficial effects on hypertiglyceridemia, hyperglycemia and hyperinsulinemia in mouse models of NIDDM and obesity (Mukherjee et al., 1997). This effect is not secondary to a variation in the weight of the animals. Furthermore, some of these rexinoids allow to determine which heterodimer is specifically involved in gene regulation

and hence these ligands provide a new tool for a better understanding of RXR action and the development of new pharmacological compounds.

Because thiazolidinediones, which are PPARγ specific ligands, are thought to exert part of their antidiabetic effect through an alteration of the fatty acid partitioning and activation of PPARγ-RXR, the inventors proposed that RXR ligands could have similar effects and be potential activators of FATP, LPL, and ACS expression. LPL, FATP, and ACS are involved in fatty acid partitioning and the levels of circulating free fatty acids depend on the expression of these genes. PPARγ activators improve glucose homeostasis and this effect may be due to the regulation of LPL (Schoonjans et al., 1996), FATP and ACS target genes. In order to confirm the implication of PPAR-RXR heterodimer in the improvement of glucose homeostasis, the regulation of LPL, FATP, and ACS genes by retinoic acid was studied. This allowed to determine which dimer was involved in this process and provided a better understanding of the regulation of lipid uptake by nuclear receptors. The PPAR-RXR heterodimer as the molecular target mediating these effects on fatty acid partitioning leading to an improvement of insulin sensitivity.

1. Material and methods

Abbreviations

RA, retinoic acid; at-RA, all-*trans* RA; 9c-RA, 9-cis RA; RAR, Retinoic acid receptor; RXR, retinoid X receptor; RXR-RE, RXR response element; TTNPB, ethyl-p-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphtyl-1-propenyl]benzoic acid; PPAR, peroxisome proliferator-activated receptor; DMSO, dimethyl sulfoxide.

Materials

at-RA was purchased from Sigma (St. Louis, Mo.) 9c-RA, TTNPB (Mangelsdorf et al., 1990) was obtained from ligand pharmaceuticals.

Cell culture and treatments

FAO cells are a well-differentiated subclone derived from the rat hepatoma H4 IIEC3 line. They were cultured at 37°C in a humidified atmosphere of 5% CO2, 95% air in Ham F-12 medium (Gibco-BRL) containing 10% fetal calf serum, penicillin (200 IU/ml) and streptomycine (50 mg/ml) according to previously published procedures (Meunier-Dumort et al., 1996). Culture medium was changed every 48 hr. Experiments were performed on subconfluent cells maintained in culture under serum free conditions. at-RA and 9c-RA were dissolved in dimethyl sulfoxide (DMSO) while oleic acid was complexed with bovine serum albumine before addition to the cells (BSA). The human hepatoma cell line HepG2 was obtained from ECACC (Porton Down, Salisbury, United Kingdom) and the mouse preadipocyte cell line 3T3-L1 from (ATCC). These cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM), supplemented with 10% fetal calf serum (LPDS-DCC), L-glutamine and antibiotics, unless stated otherwise. 3T3-L1 cells were differentiated initially by a 2 day-treatment with dexamethasone (0.1µM), isobutyl methyl xanthine (0.25mM) and insulin (0.4 µM). Subsequently, the cells were then maintained for an additional 8 days with insulin until complete differentiation. Experiments were performed on subconfluent cells maintained in culture under serum free conditions. at-RA and 9c-RA were dissolved in dimethyl sulfoxide (DMSO) while oleic acid was complexed with bovine serum albumine (BSA) before addition to the cells.

RNA analysis

RNA preparation, northern blot hybridizations, and quantification of total cellular RNA were performed as described previously (Auwerx et al., 1988). A mouse FATP cDNA probe was obtained after cloning an RT-PCR fragment from mouse adipose tissue RNA (primers ATG CGG GCT CCT GGA GCA GGA CAG CC and CTG CGT GTC AGG CAG GAT GCT CTC AGG CCC) into

pBluescript-KS. The insert was sequenced and found to be identical to the reported mouse FATP sequence. The rat ACS probe corresponds to the EcoRV restriction fragment of the rat ACS cDNA and an EcoRI fragment of the hLPL26 clone was used to measure LPLmRNA. β-actin was used as control probe.

Isolation of nuclei and transcriptional rate assay

Nuclei were prepared from FAO and 3T3-L1 differentiated cells and treated either with 9c-RA or vehicle. Transcription run-on assays were performed as described by Nevins (Nevins, 1987). Equivalent counts of nuclear RNA labeled with [α-^{32}P]UTP (3000 Ci/mmol) were hybridized for 36 hr at 65°C to 5 µg of FATP, ACS, GAPDH and vector DNA (pBluescript) immobilized on Hybond-C Extra filters (Amersham). After hybridization, filters were washed at room temperature for 10 min in 0.5xSSC and 0.1% SDS and twice at 65°C for 30 min and subsequently exposed to X-ray film (BIOMAX-MS, Kodak). Quantitative analysis was performed by scanning densitometry (BioRad GS670 densitometer).

Preparation of albumin-bound fatty acids and Fatty acid uptake assay

Radiolabeled ^{14}[C] oleate fatty acid was added to water at 40°C. Albumin (BSA; fraction V, fatty acid free, Sigma, St Louis, Mo) was then added from a concentrated stock (20 g/100 ml) to give a final molar ratio of 1/1 by gentle mixing. 2 X Hank's solution was added to obtain a 1 X final solution. Incubation was carried out at 37°C for 45 minutes.

The measurement of uptake of 1^{14}[C]-labeled oleate (about 50 mCi/mmol, NEN, Boston, MA) was carried out in 24 or 6 well plates with 10^6 cells / ml of medium. Before treatment, the cells were washed with 1X Hank's solution. BRL 49653 (100 - 250 nM) and fenofibric acid (100 - 250 µM) and the other retinoids (dose range from 10^{-9} M to 10^{-6} M) were added in fresh DMEM medium containing 10% FCS. After 48 h of treatment, cells were washed with Hank's solution and incubated for one additional hour in serum-free, glucose-

free medium. Cells were then washed once at 37°C and twice at 23 °C with 1X Hanks solution containing BSA. Hank's solution without BSA was then added before the assay. A volume corresponding to 1µCi of ^{14}[C]-oleate albumin-bound solution was added in each well and cells were incubated for 1 min at room temperature. Incubation was stopped after 1 min with 3 washes of ice cold 1X Hank's solution without BSA. A complementary experiment has been performed to verify whether a specific cell surface binding of ^{14}[C]-oleate could interfere with the assay. For this second assay, the cells were washed under more stringent conditions in 1X Hank's solution containing 0.5% BSA. Cells were then lysed in 400 µl of 0.1% SDS solution. The lysate was counted for 5 min with 4 ml of scintillation solution. Assay was performed on triplicate points.

Results

1.9c-RA induces FATP and ACS mRNA levels in the hepatoma cells FAO and HepG2

In order to determine whether FATP and ACS genes were regulated at transcriptional level by retinoic acid, a dose-response experiment was performed. FAO cells were cultured for 6 hr with increasing doses of 9c-RA (10^{-8} to 10^{-4} M) whereas control cells were incubated with vehicle alone (0,1% v/v DMSO). Incubation with 9c-RA resulted in a dose-dependent increase of both FATP and ACS mRNA levels in this hepatic cell line (figure 10A). A maximal increase for FATP (>9-fold) and ACS (14-fold) was observed with a dose of 10^{-6}M 9c RA. FATP and ACS m RNA are also upregulated by at-RA but to a lesser extent that 9c-RA (data not shown).

Induction of FATP and ACS mRNA levels in the human hepatoma cell line HepG2 was analyzed. 9c-RA induced FATP and ACS mRNA after 24 hr (dose 10^{-8} M) (figure 10B). However, no effect of at-RA could be detected (data not shown).

2. 9c-RA induces FATP and ACS mRNA levels in differentiated Caco2 and 3T3-L1 cells.

Since adipose tissue is an important insulin-sensitive tissue, it was interesting to know whether retinoids also regulate FATP and ACS gene expression in mouse adipose cell line, 3T3-L1. 3T3-L1 cells were therefore completely differentiated and then treated with 9c-RA (10^{-9} to 10^{-6} M). 9c-RA induces FATP and ACS mRNA expression in a dose-dependent manner. Maximum increase was observed at a concentration of 10^{-6} M 9c-RA (figure 11A).

The expression of FATP and ACS induction was next compared between Fao hepatoma cells and the colon adenocarcinoma cells Caco2. Both FATP and ACS gene expressions are increased after 6 hr of treatment with 9c-RA at 10^{-6} M. However, higher levels of induction are nevertheless observed in FAO cells (figure 11B).

3. The induction of FATP and ACS gene expression by 9c-RA is at the transcriptional level.

Nuclear run-on analysis was next carried out to determine whether the induction of this expression by 9c-RA was a direct consequence of enhanced gene transcription. FAO cells were therefore treated for 2 hr with 9c-RA (10^{-6} M) after 18 hr of culture in serum-deprived medium, whereas 3T3-L1 cells were treated for 24 hr with 9c-RA (10^{-6} M) in normal medium without serum deprivation. FAO metabolism is greatly affected with lipids, serum deprived conditions allow to observe a strong effect which couldn't be detected in normal conditions. Controls were treated with vehicle only. Transcription rates for the FATP and ACS genes were induced 2-fold and 2.8-fold respectively in FAO cells and 4- and 3-fold respectively in differentiated 3T3-L1 cells. In the retinoic acid treated cells, transcription of the GAPDH gene was not affected in neither of the two cell lines.

4. Rexinoids, but not RAR agonists, induce FATP, ACS and LPL gene expression in differentiated 3T3-L1 cells.

To determine whether the effect of retinoids on the regulation of FATP and ACS gene expression was mediated by RAR or RXR, 3T3-L1 differentiated cells were treated with 9c-RA, a panagonist of both RAR or RXR, or at-RA and TTNPB which are specific agonists of RAR. No activation of LPL, FATP and ACS gene expression was detectable after 24 hr of treatment with at-RA or TTNPB. In contrast, a strong dose-dependent induction of both LPL, FATP and ACS was observed 24 hr after treatment with 9c-RA, a RXR agonist. Maximum induction occurred at 10^{-6} M 9c-RA (4-fold and 3-fold for LPL, FATP and ACS respectively). Interestingly, no effect of non of the retinoids was observed on non-differentiated 3T3-L1 preadipocyte cells (figures 13, 14 and 15).

RXR agonists, but not RAR agonists, induce oleate uptake in differentiated 3T3-L1 cells.

To establish whether the induction of the FATP-1 and ACS was accompanied by a concommitant increase in fatty acid uptake into the cells, we measured ^{14}[C] oleate uptake in differentiated 3T3-L1 cells exposed to increasing doses of prototypic retinoid, i.e., TTNPB, an RAR agonist (dose range 10^{-9} M to 10^{-6} M). No effect on ^{14}[C] oleate uptake was observed after TTNPB confirming that this effect was specific for the rexinoids (figure 16).

Discussion

These results demonstrate that RXR ligands control both FATP and ACS gene expression in several cell types. In the liver, FATP and ACS have been shown to be strongly upregulated by PPARα activators (Schoonjans et al., 1995; Martin et al., 1997). These distinct transcriptional effects are mediated by

PPARα interacting with responsive elements in the promoter regions of these genes. As PPARα effects are mediated through activation of the PPARα-RXR heterodimer, it was not surprising to observe a strong induction of FATP and ACS expression by specific RXR activators. Expression of FATP and ACS are co-induced by 9c-RA in the liver and in intestine. Free fatty acids generated by LPL are avidly taken up by these tissues and are converted in metabolic active acyl-CoA derivatives to sustain a high level of β-oxydation. The co-induction of FATP and ACS by 9c-RA, which resembles the induction of these genes by fibrates, indicates that 9c-RA and fibrates have a similar metabolic effect on rat and human hepatoma cell line (Kliewer et al.). These data demonstrate that the RA pathway is implicated in the regulation of lipid metabolism and underline the powerful action of specific RXR agonists on the uptake and metabolism of long-chain fatty acids in the liver.

In adipose tissue, a second insulin-sensitive tissue, FATP and ACS are also co-regulated by 9c-RA in a dose-dependent manner. PPARγ is the predominant in this tissue and PPARγ activators have been shown to induce both FATP and ACS expression. Hence, the induction of FATP and ACS expression by RXR agonists in the context of PPARγ-RXR heterodimer suggests that RXR is also here an active heterodimer partner.

The stimulatory effects of 9c-RA on FATP and ACS gene expression in both tissue implicate the RA pathway as an important regulation pathway in liver and adipose tissue where retinoids have a significant physiological role in fatty acid metabolism. Since FATP and ACS genes are implicated in fatty acid partitioning, it can be concluded that the distribution of fatty acids is affected in response to 9c-RA. This effect of rexinoids on fatty acid partitioning is different from the effects observed with PPAR activators and depends on the relative expression of the different RXRs and PPARx (Braissant et al., 1994; Lemberger et al. 1996; Auboeuf et al., 1997). RXR is present in both liver and adipose tissue and this pattern of expression determines the tissue specific effects of RA activation. In contrast, activity of PPARα agonists is mostly limited to the

liver, the prime site of expression of PPARα. Furthermore, the fact that the co-regulation of LPL, FATP and ACS genes by 9c-RA, TTNPB and at-RA depend on the differentiated state of 3T3-L1 cells points to the importance of PPArg in this process. In preadipocyte cells PPARγ is expressed at low levels and its expression increases upon adipocyte differentiation. The absence of a retinoic acid response in differentiated 3T3-L1 cells expressing almost no PPARγ suggest that PPARγ is an obligatory partner of RXR in the retinoid-dependent regulation of LPL, FATP and ACS genes in adipose tissue. Hence, due to its more general expression, it is expected that the effects of rexinoids are more generalized than the effects of the more tissue-restricted PPARα (liver) and PPARγ (adipose tissue) agonists. In fact, rexinoids effects on gene expression should resemble the effects of a combined PPARα and γ agonist. This effect is direct on the FATP gene and suggests the presence of a response element like it has been demonstrated in ACS and LPL gene (Schoonjans et al., 1996) but also and recently in FABP gene (Poirier et al., 1997).

It seems that rexinoids, the new term used to specify RXR selective ligands collaborate with PPAR ligands to control the expression of FATP and ACS genes involved in fatty acid metabolism. The beneficial effect of PPARγ activators on glucose homeostasis has been previously shown by us to be in part due to a redistribution of fatty acids towards adipose tissue with a relative depletion of fatty acids in the muscle (Martin et al., 1998). As known since Randle's work in the 60 (Randle et al., 1963; Randle et al., 1964), depletion of muscle fatty acid content will result in an improvement of glucose homeostasis. Hence, it is tempting to speculate that the improvements of glucose homeostasis observed with rexinoids are similarly linked to an altered partitioning of fatty acids. In view of the distinct tissue distribution of RXR expression, induction of FATP and ACS by RXR ligands might not have the same tissue-specificity as that of PPARα and PPARγ activators and could contribute to a different tissue partitioning of fatty acids. These agents provide hence an alternative way to regulate the expression of genes implicated in fatty

acid distribution among the different tissues.

This study demonstrates that RXR ligands regulate the expression of LPL, FATP and ACS genes in several cell types. The retinoic acid pathway is an important signalling pathway for the regulation of genes which are implicated in fatty acid supply. Our results suggest that the PPAR-RXR complex is the molecular target by which rexinoids regulate FATP and ACS gene expression. Furthermore, they define FATP and ACS as new target genes in the RXR-dependent signalling pathway. The observation that both rexinoids and peroxisome proliferators stimulate the transcription of these genes implicated in lipid metabolism, suggest that rexinoids may act as an hypolipidemic and hypoglycemic agent through activation of PPAR-RXR complex and are consistent with the hypothesis that the heterodimer PPAR-RXR heterodimer is the molecular target for the improvement of insulin sensitivity.

TTNPB, which is an activator of RAR in the context of RXR-RAR heterodimer, has no effect on FATP-1 and ACS. The combined results of the studies using these synthetic retinoids implicates the PPAR-RXR heterodimer as the molecular target of the regulation of FATP-1 and ACS by retinoic acids. Since PPAR and RXR are both active components of this heterodimer and since we have previously demonstrated that the FATP-1 and ACS genes are both regulated by PPAR activator (Martin, G. et al (1997) J. Biol. Chem. 272:28210-7), it appears that rexinoids could cooperate synergistically with PPAR ligands in the control of the expression of these two genes involved in fatty acid metabolism. Furthermore, the fact that the coregulation of FATP-1 and ACS genes by rexinoids depends on the differentiated state of 3T3-L1 cells points to the importance of PPARγ in this process. In preadipocyte cells PPARγ is expressed at low levels and its expression increases upon adipocyte differentiation. The absence of a rexinoid response is undifferentiated 2T3-L1 cells, expressing almost no PPARγ, hence also suggest that PPARγ is an oligatory partner of RXR in the retinoid-dependent regulation of FATP-1 and ACS genes in adipose tissue.

Example 3

Expression of FATP in human tissues

In order to determine whether the expression of FATP was ubiquitous or limited to some tissues, hybridization experiments were performed with a human probe radioactively labeled on a commercial Northern Blot (Clontech) containing mRNA of 8 human tissues. These tissues are heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas tissues (figure 14). The expression is very high in the skeletal muscle, high in heart and pancreas, medium in brain, weak in placenta, liver, and very weak in kidneys. The human mRNA corresponding to FATP has a size of 4.4 kb. The human β-actin probe has been used for the normalization and the top signal represents the ubiquitously expressed isoform, whereas skeletal and cardiac muscle express a specific β-actin isoform.

The commercial Northern Blot did not contain adipose tissue probably due to difficulties in performing its extraction. Therefore, a supplementary Northern Blot analysis was performed on various human cell lines and adipose tissue. The expression of FATP was analyzed. FATP was very highly expressed in adipose tissue. To a weaker extent, FATP was expressed in hepatocyte and monocytic cell lines. The expression was detectable in chorionic carcinoma cells (Jeg3) and human colon cells (Caco2).

The high level of FATP mRNA in the skeletal muscle has been checked on several skeletal muscular tissues and the level of expression of the FATP mRNA in this tissue has been compared with the level in sub-cutaneous or visceral adipose tissues, liver and colon tissues (figure 17).

Interestingly, the expression level in the human muscle (lines 1, 2, and 3) has been confirmed at a level which is close to the level observed in adipose tissue,

which was not the case when the same experiments were performed on rodents. The expression of FATP is very high in the adipose tissue. In normal patients, the adipose tissue is sub-cutaneous. In patients suffering from obesity, the adipose tissue is visceral and sub-cutaneous. In these patients, the expression of FATP is higher at the level of the sub-cutaneous tissue.

FATP is thus expressed ubiquitously in human tissues, with a predominant expression in adipose tissue, muscle and heart.

As a transport protein, FATP would permit transportation of fatty acids in the adipose tissue in nutrition periods and would be involved in efflux of said fatty acids in fast periods. Furthermore based on the observation that FATP is highly expressed in skeletal muscle, it is concluded that FATP is very important as energy source for a tissue which is known to use mainly glucose in normal conditions. This result emphasizes a competition between glucose and long-chain fatty acids for the muscular activity.

The third tissue which expressed a high level of FATP is heart. This expression is not influenced by nutrition. In brain tissue the level of FATP is significant. In addition to its activity as energy source, FATP could participate in the incorporation of lipids for synthesis of axial membranes and myelin. In the pancreas, FATP could be the specific transport protein of long-chain fatty acids messengers essential for the regulation of the production of insulin by the pancreas.

It is especially noted that the level of FATP is especially high in tissues which are sensitive to insulin such as adipose tissues and muscle.

Western bl t analysis f FATP.

Cells and tissues were homogenized in a lysis buffer of PBS containing 1% Triton X-100 (Sigma, St Louis, MO). Tissues were homogenized with a polytron

in extraction buffer containing PBS and 1% NP-40, 0,5 % sodium deoxycholate, 0,1% SDS (Sigma, St Louis, MO). In the presence of a fresh cocktail of protease inhibitors (ICN, France) (100 µg/ml AEBSF, 5 mg/ml EDTA, 1µg/ml leupeptin, 1 µg/ml pepstatin). Protein extracts were obtained by centrifugation of the lysate at 4°C and then concentration was measured with the Biorad DC Protein colorimetric assay system (Biorad, France).

Protein (100 µg) were separated on a 10% polyacrylamide gel according to Laemmli [Laemmli, 1970 #1541], transferred to nitrocellulose membrane as described by Towbin [Towbin, 1979#1542] (Amersham, France), and blocked overnight in blocking buffer (20 mM Tris, 100 mM NaCl, 1% Tween-20, 10% skm milk). Filters were first incubated 4 hours at 21°C with rabbit IgG anti-mFATP (10 mg/ml) developed against a FATP peptide, corresponding to amio acids from human FATP-1, and next for 1 hour at 21°C with a goat anti-rabbit IgG (whole molecule) peroxidase conjugate (Pasteur Diagnostic Sanofi, France) diluted at 1/5000. The complex was visualized by chemiluminescence using with 4-chloro-1-naphtol as reagent according to the manufacturer's protocol (ECL, Amersham, France).

Northern blot analysis of different human cell lines, such as NCL-H295, THP-1, Caco2, chorionic cells and hepatoma cells shows that hFATP-1 can be detected in all these cells but to a relatively lower extent than in human adipose tissue (figure 18). hFATP1 mRNA is relatively will expressed in Caco2 cells and in cells of hepatic origin.

References

Auboeuf, D. et al, (1997) "Tissue distribution and quantification of the expression of PPARs and LXRα in humans: no alterations in adipose tissue of obese and NIDDM patients." Diabetes 48: 1319-27.

Auwerx, J. et al, (1988) "Transcriptional activation of the lipoprotein lipase and apolipoprotein E genes accompanies differentiation in some human macrophage-like cell lines." Biochemistry 27: 2651-5.

Boden, G., et al., (1994) "Mechanisms of fatty acid-induced inhibition of glucose uptake." J. Clin. Invest. 93: 2438-46.

Boehm, M.F., et al., (1994) "Synthesis and structure activity of novel retinoid X receptor selective retinoids"., J. Med. Chem. 37: 2930-41.

Boehm, M.F., (1995) et al., "Design and synthesis of potent retinoid X receptor selective ligands that induce apoptosis in leukemia cells." J. Med. Chem. 38; 3146-3155.

Braissant, O. and W. Wahli (1994). Etude de l'expression des récepteurs nucléaires PPARs chez le rat adulte., Université de Lausanne, Suisse.

Chomczynski, P. et al (1987). "Single step method for RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extraction." Anal. Biochem. 162: 156-9.

Chou et al. (1974). "Conformational parameters for amino acids in helical, beta-

sheet, and random coil regions calculated from proteins." Biochemistry 13: 211-22.

Deschatrette, J. et al. (1974). "Characterization of differentiated and dedifferentiated clones from a rat hepatoma". Biochimie 56: 1603-12.

Evans, R.M. (1988). " The steroid and thyroid hormone receptor superfamily." Science 240: 889-95.

Faergeman et al. (1997). "Disruption of the Saccharomyces cerevisiae homologue to the murine fatty acid trasnport protein impairs uptake and growth on long chain fatty acids." J. Biol. Chem. 272: 8531-8.

Fajas et al. (1997). "Cycle sequencing on large DNA templates." Biotechniques 23: 1034-6.

Francke et al. (1978). "Quantitative analysis of high resolution trypsin-giemsa bands on human promethaphase chromosomes." Hum. Genet. 45: 137-65.

Garnier et al. (1978). "Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins." J. Mol. Biol. 120: 97-120.

Geffroy et al. (1995). "Localization of the human ob gene to chromosome 7q32 by fluorescence in situ hybridization." Genomics 28: 603-4.

Giguere, V. (1994). "Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signalling." Endocrine Reviews 15: 61-79.

Hertz, R. and J. Bar-Tana (1992). "Induction of peroxisomal b-oxidation genes by retinoic acid in cultured rat hepatocytes." Biochem. J. 281: 41-3.

Higgins, C.F. (1994). "Flip-flop : the transmembrane translocation of lipids." Cell 79:393-5.

Kliewer, S.A., et al (1992). "Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors." Nature 358: 771-4.

Kyte et al. (1982). "A simple method for displaying the hydropathic character of a protein." J. Mol. Biol. 157: 105-32.

Lala, D.S., et al (1996) "Activation of specific RXR heterodimer by an antagonist of RXR homodimers." Nature 383:450-3

Leid, M., et al (1992). "Mutiplicity generates diversity in the retinoic acid signalling pathways." Trends in Biochemical Sciences 17: 427-33.

Lemberger, T. O. et al (1996). "PPAR tissue distribution and interactions with other hormone-signaling pathways." Ann. N.Y. Acad.Sci. 804:231-51.

Mangelsdorf, D.J., et al (1990). "Nuclear receptor that identifies a novel retinoic acid response pathway." nature 345: 224-9.

Martin, G. et al (1997). "Coordinate regulation of the expression of the fatty acid transport protein (FATP) and acyl CoA synthetase genes by PPARα and PPARγ activators." J. Biol. Chem. 272: 28210-7.

Martin, G. et al. (1998). " PPARγ activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes." Atherosclerosis.

Meunier-Dumort, C., et al. (1996) "Up-regulation of the expression of the gene for liver fatty acid-binding protein by long-chain fatty acids." Biochem. J. 319: 483-7.

Negrel et al. (1978). "Establishment of preadipocyte clonal cell line from epididymal fat pad of ob/ob mouse that responds to insulin and to lipolytic hormones." Proc. Natl. Acad. Sci. USA 75: 6054-8.

Nevins, J.R. (1987). "Isolation and analysis of nuclear RNA." Methods in Enzymology 152: 234-41.

Poirier, H. et al (1997). "9-cis-retinoic acid enhances fatty acid induced expression of the liver fatty acid-binding protein gene." FEBS Lett. 412: 480-4.

Randle, P.J., et al (1963). "The glucose-fatty acid cycle; its role in insulin sensitivity and metabolic disturbances of diabetes mellitus." Lancet I: 785-9.

Randle, P.J., et al. (1963) "Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies, and pyruvate, and of alloxan-diabets and starvation on the uptake of metabolic fate of glucose in rat heart and diaphragm musceles." Biocehm. J. 93: 652-65.

Reddy, J.L. and N.D. Lalwani (1983). "Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans." CRC Crit. Rev. Toxicol. 12: 1-58.

Sambrook et al. (1989). Molecular cloning; a laboratory manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press.

Schaffer, J.E. and H.F. Lodish (1994). "Expression cloning and characterization of a novel long-chain fatty acid transport protein." Cell 79: 427-36.

Schoonjans, K., et al. (1995). "Induction of the Acyl-Coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter." J. Biol. Chem. 270: 19269-76.

Tsai, M.-J. and B. O'Malley (1994). "Molecular mechanisms of action of steroid/thyroid receptor superfamily members." Annual Reviews of Biochemistry 63:451-86.

von Heijne, G. et al. (1986). "A new method for predicting signal sequence cleavage sites." Nucl. Acids Res. 14: 4683-90.

Wu et al. (1994). "Establishment and characterization of differentiated nontransformed hepatocyte cell lines derived from mice transgenic for transforming growth factor α." Proc. Natl. Acad. Sci. USA, 91: 674-8.

CLAIMS

1. Nucleotide sequence which comprises a sequence involved in the expression of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of Figure 2 or Figure 5.

2. Nucleotide sequence according to claim 1, which comprises a sequence encoding the human FATP, corresponding to or comprising the nucleotide sequence of Figure 1 or Figure 3.

3. Nucleotide sequence according to Claim 2, which is the nucleotide sequence of Figure 1 or Figure 3.

4. Nucleotide sequence according to Claim 2, which comprises an ORF sequence located between nucleotide 23 (ATG) and nucleotide 1963 (TGA) of Figure 1 or Figure 3.

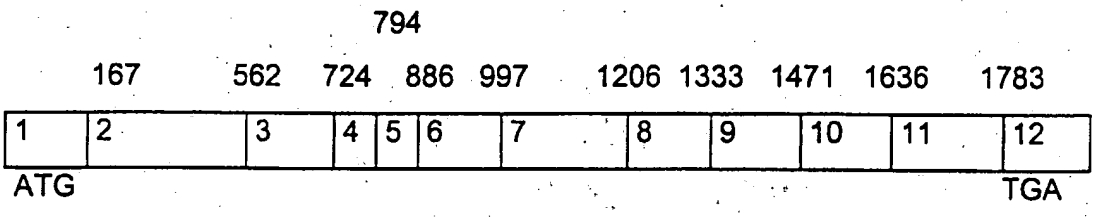
5. Nucleotide sequence hybridizing in high stringency conditions, with a probe comprising 50 to 300 nucleotides including at least 6, preferably at least 9 nucleotides from the following sequence :
CGGGGAGACGGGACGTGAAGGG.

6. Nucleotide sequence according to anyone of claims 1 to 5 which is a genomic DNA.

7. Nucleotide sequence according to anyone of Claims 1 to 6, which is the gene coding for the hFATP.

8. Nucleotide sequence according to anyone of Claims 1 to 3 or 6 to 7, which comprises a transcription initiation site 61 bp upstream from the ATG codon.

9. Nucleotide sequence according to anyone of Claims 6 to 8, which contains 12 exons and 11 introns having the following structure:



10. Nucleotide sequence according to claim 9, which comprises the nucleotide sequence of Figure 4.

11. Nucleotide sequence according to anyone of claims 1 to 10, which comprises sequences involved in the regulation of the expression of the gene coding for the human FATP.

12. Nucleotide sequence according to anyone of Claims 1 to 11, which sequence either specifically hybridizes with one or both of the primers having one of the following sequences or is the amplification product obtained with the following sequences, or hybridizes in high stringency conditions with said amplification product :

AAGGTCAATGAGGACACAATGG,

CGAGTAGGTAGTGATCGTGCAG.

13. Nucleotide sequence according to anyone of claims 1 to 12, which is a genomic sequence coding for FATP and which hybridizes in high stringency conditions with a probe containing around 200 bp and being preferably derived from the sequence of exon 2, said genomic nucleotide sequence comprising a sequence involved in the regulation of the gene coding for the FATP.

14. Nucleotide sequence which is a mRNA as obtained by transcription of a genomic nucleotide sequence coding for the hFATP according to anyone of Claims 6 to 13.

15. Nucleotide sequence according to anyone of Claims 1 to 13 which is the RNA sequence transcribed from the nucleotide sequence of figure 1 or figure 3 having 2 kb, or an RNA sequence including a nucleotide sequence of 4,4 kb hybridizing in high stringency conditions with the sequence of figure 1 or

figure 3.

16. cDNA as obtained by reverse transcription of the mRNA according to Claim 14 or 15.

17. Polynucleotide which is selected among the following sequences:

- AAGGTCAATGAGGACACAATGG
- CGAGTAGGTAGTGATCGTGCAG
- a sequence comprising or corresponding to sequences involved in the regulation of the expression of the gene encoding the hFATP,
- any fragment derived from the nucleotide sequences disclosed in figures 1, 3 or 4, for instance by deletion mutation or insertion provided the essential biological properties of the native sequences are maintained.

18. Vector comprising, inserted in a site not essential for its replication, a nucleotide sequence according to anyone of claims 1 to 16.

19. Vector according to Claim 18, which further comprises a reporter sequence such as the CAT gene or the luciferase gene.

20. Vector according to Claim 19, wherein the expression of the reporter gene is under the control of an heterologous regulator region.

21. Recombinant cell which comprises a nucleotide sequence according to anyone of claims 1 to 16 or a vector according to anyone of Claims 18 to 20.

22. Recombinant cell according to Claim 21 which is a eucaryotic cell.

23. Human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of figure 2 or figure 5.

24. Human FATP according to Claim 23, which has an isoelectric point of 8.5 and which replies to the aminoacid sequence of Figure 2 or Figure 5.

25. Human FATP which is encoded by a nucleotide sequence according to anyone of Claims 1 to 16.

26. Human FATP which is devoid from its signal peptide.

27. Human FATP according to anyone of claims 23 to 26, which is

glycosylated.

28. Antibodies specifically directed against hFATP according to anyone of Claims 23 to 27.

29. Composition for the therapeutic modulation of the intracellular level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

30. Composition for the therapeutic modulation of the blood level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

31. Composition for the treatment of a pathological state associated with a deficient regulation of the intracellular level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

32. Composition according to claim 30 or 31, which comprises an inhibitor of the expression of the hFATP gene.

33. Composition according to claim 30 or 31, which comprises a compound capable of enhancing the expression of the hFATP gene.

34. Composition according to claim 33 for the treatment of cardiomyopathies or diabetes.

35. Composition according to claim 32 for the treatment of obesity.

36. Method for the screening of the effect of a determined compound, on the expression of the hFATP protein in determined cells, which method comprises the step of detecting the transcription of the mRNA in the cells or cell extracts, after contacting the cells with said determined compound, in conditions enabling the interaction between said compound and said cells.

37. Method according to claim 36, for the screening of the effect of a determined compound on the regulation of the expression of the hFATP protein in cells, which comprises:

a) measuring the level of transcription of the mRNA in cells or cell

extracts, wherein the cells have previously been contacted with the determined compound, in conditions enabling the interaction of said cells and said determined compound;

b) measuring the level of transcription of the mRNA in the same cells species as in step a) or on extracts of these cells, wherein these cells have not been previously contacted with the assayed compound;

c) comparing the level of transcription obtained in steps a) and b).

38. Method according to claim 36 or 37, wherein the cells used are selected among the group of liver cells, heart cells, adipose tissue cells and skeletal muscle cells.

FIG. 1A

```
1
CGGGGAGACGGGACGTGAAGGGATGCGGGCTCCGGGTGCG    hFATP1 -UTR
                      ATGCGGGCTCCGGGTGCG    hFATP' -UTR
1

41
GGCGCGGCCTCGGTGGTCTCGCTGGCGCTGTTGTGGCTGC    hFATP1 -UTR
GGCGCGGCCTCGGTGGTCTCGCTGGCGCTGTTGTGGCTGC    hFATP' -UTR
41

81
TGGGGCTGCCGTGGACCTGGAGCGCGGCAGCGGCGCTCGG    hFATP1 +UTR
TGGGGCTGCCGTGGACCTGGAGCGCGGCAGCGGCGCTCGG    hFATP' +UTR
81

121
CGTGTACGTGGGCAGCGGCGGCTGGCGCTTCCTGCGCATC    hFATP1 +UTR
CGTGTACGTGGGCAGCGGCGGCTGGCGCTTCCTGCGCATC    hFATP' +UTR
121

161
GTCTGCAAGACCGCGAGGCGAGACCTCTTCGGTCTCTCTG    hFATP1 +UTR
GTCTGCAAGACCGCGAGGCGAGACCTCTT-----------    hFATP' +UTR
161

201
TGCTGATCCGCGTGCGCCTGGAGCTGCGGCGGCACCAGCG    hFATP1 +UTR
----------------------------------------    hFATP' +UTR
189

241
TGCCGGCCACACCATCCCGCGCATCTTTCAGGCGGTAGTG    hFATP1 +UTR
------------------------------GCGGTAGTG    hFATP' +UTR
189

281
CAGCGACAGCCCGAGCGCCTGGCGCTGGTGGATGCCGGGA    hFATP1 +UTR
CAGCGACAGCCCGAGCGCCTGGCGCTGGTGGATGCCGGGA    hFATP' +UTR
199

321
CCGGCGAGTGCTGGACCTTTGCGCAGCTGGACGCCTACTC    hFATP1 +UTR
CCGGCGAGTGCTGGACCTTTGCGCAGCTGGACGCCTACTC    hFATP' -UTR
239

361
CAATGCGGTAGCCAACCTCTTCCGCCAGCTGGGCTTCGCG    hFATP1 -UTR
CAATGCGGTAGCCAACCTCTTCCGCCAGCTGGGCTTCGCG    hFATP' -UTR
279
401
CCGGGCGACGTGGTGGCCATCTTCCTGGAGGGCCGGCCGG    hFATP1 -UTR
CCGGGCGACGTGGTGGCCATCTTCCTGGAGGGCCGGCCGG    hFATP' -UTR
319

441
AGTTCGTGGGGCTGTGGCTGGGCCTGGCCAAGGCGGGCAT    hFATP1 -UTR
AGTTCGTGGGGCTGTGGCTGGGCCTGGCCAAGGCGGGCAT    hFATP' -UTR
359
```

FIG. 1B

481
GGAGGCCGCGCTGCTCAACGTGAACCTGCGGCGCGAGCCC hFATP1 +UTR
GGAGGCCGCGCTGCTCAACGTGAACCTGCGGCGCGAGCCC hFATP' +UTR
399

521
CTGGCCTTCTGCCTGGGCACCTCGGGCGCTAAGGCCCTGA hFATP1 +UTR
CTGGCCTTCTGCCTGGGCACCTCGGGCGCTAAGGCCCTGA hFATP' +UTR
439

561
TCTTTGGAGGAGAAATGGTGGCGGCGGTGGCCGAAGTGAG hFATP1 +UTR
TCTTTGGAGGAGAAATGGTGGCGGCGGTGGCCGAAGTGAG hFATP' +UTR
479

601
CGGGCATCTGGGGAAAAGTTTGATCAAGTTCTGCTCTGGA hFATP1 +UTR

641
GACTTGGGGCCCGAGGGCATCTTGCCGGACACCCACCTCC hFATP1 +UTR

681
TGGACCCGCTGCTGAAGGAGGCCTCTACTGCCCCCTTGGC hFATP1 +UTR

721
ACAGATCCCCAGCAAGGGCATGGACGATCGTCTTTTCTAC hFATP1 +UTR

761
ATCTACACGTCGGGGACCACCGGGCTGCCCAAGGCTGCCA hFATP1 +UTR

801
TTGTCGTGCACAGCAGGTACTACCGCATGGCAGCCTTCGG hFATP1 +UTR

841
CCACCACGCCTACCGCATGCAGGCGGCTGACGTGCTCTAT hFATP1 +UTR

881
GACTGCCTGCCCCTGTACCACTCGGCAGGAAACATCATCG hFATP1 +UTR

921
GCGTGGGGCAGTGTCTCATCTATGGGCTGACAGTCGTCCT hFATP1 +UTR

961
CCGCAAGAAATTCTCGGCCAGCCGCTTCTGGGACGACTGT hFATP1 +UTR

1001
ATCAAGTACAACTGCACGGTGGTTCAGTACATCGGGGAGA hFATP1 -UTR

1041
TCTGCCGCTACCTGCTGAAGCAGCCGGTGCGCGAGGCGA hFATP1 -UTR

FIG. 1C

```
1081
GAGGCGACACCGCGTGCGCCTGGCGGTGGGGAACGGGCTG    hFATP1 -UTR

1121
CGTCCTGCCATCTGGGAGGAGTTCACGGAGCGCTTCGGCG    hFATP1 -UTR

1161
TACGCCAAATCGGGGAGTTCTACGGCGCCACCGAGTGCAA    hFATP1 -UTR

1201
CTGCAGCATTGCCAACATGGACGGCAAGGTCGGCTCCTGT    hFATP1 -UTR

1241
GGTTTCAACAGCCGCATCCTGCCCCACGTGTACCCCATCC    hFATP1 -UTR

1281
GGCTGGTGAAGGTCAATGAGGACACAATGGAGCTGCTGCG    hFATP1 -UTR

1321
GGATGCCCAGGGCCTCTGCATCCCCTGCCAGGCCGGGGAG    hFATP1 -UTR

1361
CCTGGCCTCCTTGTGGGTCAGATCAACCAACAGGACCCGC    hFATP1 -UTR

1401
TGCGCCGCTTCGATGGCTATGTCAGCGAGAGCGCCACCAG    hFATP1 -UTR

1441
CAAGAAGATCGCCCACAGCGTCTTCAGCAAGGGCGACAGC    hFATP1 -UTR

1481
GCCTACCTCTCAGGTGACGTGCTAGTGATGGATGAGCTGG    hFATP1 -UTR

1521
GCTACATGTACTTCCGGGACCGTAGCGGGGACACCTTCCG    hFATP1 -UTR

1561
CTGGCGAGGGGAGAACGTCTCCAACACCGAGGTGGAGGGC    hFATP1 -UTR

1601
GTGCTGAGCCGCCTGCTGGGCCAGACAGACGTGGCCGTCT    hFATP1 -UTR

1641
ATGGGGTGGCTGTTCCAGGAGTGGAGGGTAAGGCAGGGAT    hFATP1 -UTR

1681
GGCGGCCGTCGCAGACCCCCACAGCCTGCTGGACCCCAAC    hFATP1 -UTR

1721
GCGATATACCAGGAGCTGCAGAAGGTGCTGGCACCCTATG    hFATP1 -UTR
```

FIG. 1D

```
1761
CCCGGCCCATCTTCCTGCGCCTCCTGCCCCAGGTGGACAC     hFATP1 +UTR

1801
CACAGGCACCTTCAAGATCCAGAAGACGAGGCTGCAGCGA     hFATP1 +UTR

1841
GAGGGCTTTGACCCACGCCAGACCTCAGACCGGCTCTTCT     hFATP1 +UTR

1881
TCCTGGACCTGAAGCAGGGCCACTACCTGCCCTTAAATGA     hFATP1 +UTR

1921
GGCAGTCTACACTCGCATCTGCTCGGGCGCCTTCGCCCTC     hFATP1 +UTR

1961
TGAAGCTGTTCCTCTACTGGCCACAAACTCTGGGCGTGGT     hFATP1 +UTR

2001
GGGAGAGGCCAGCTTGAGCCAGACAGCGCTGCCCAGGGGT     hFATP1 +UTR

2041
GGCCGCCTAGTACACACCCACCTGGCCGAGCTGTACCTGG     hFATP1 +UTR

2081
CACGGCCCATCCTGGACTGAGAAACTGGAACCTCAGAGGA     hFATP1 +UTR

2121
ACCCGTGCCTCTCTGCTGCCTTGGTGCCCCTGTGTCTGCC     hFATP1 +UTR

2161
TCCTCTCCCTGCTTTTCAGCCTNTGTCTCCTTCCATCCNT     hFATP1 +UTR

2201
GTCCCTGTNTGGCCTTAACCCG                       hFATP1 +UTR
```

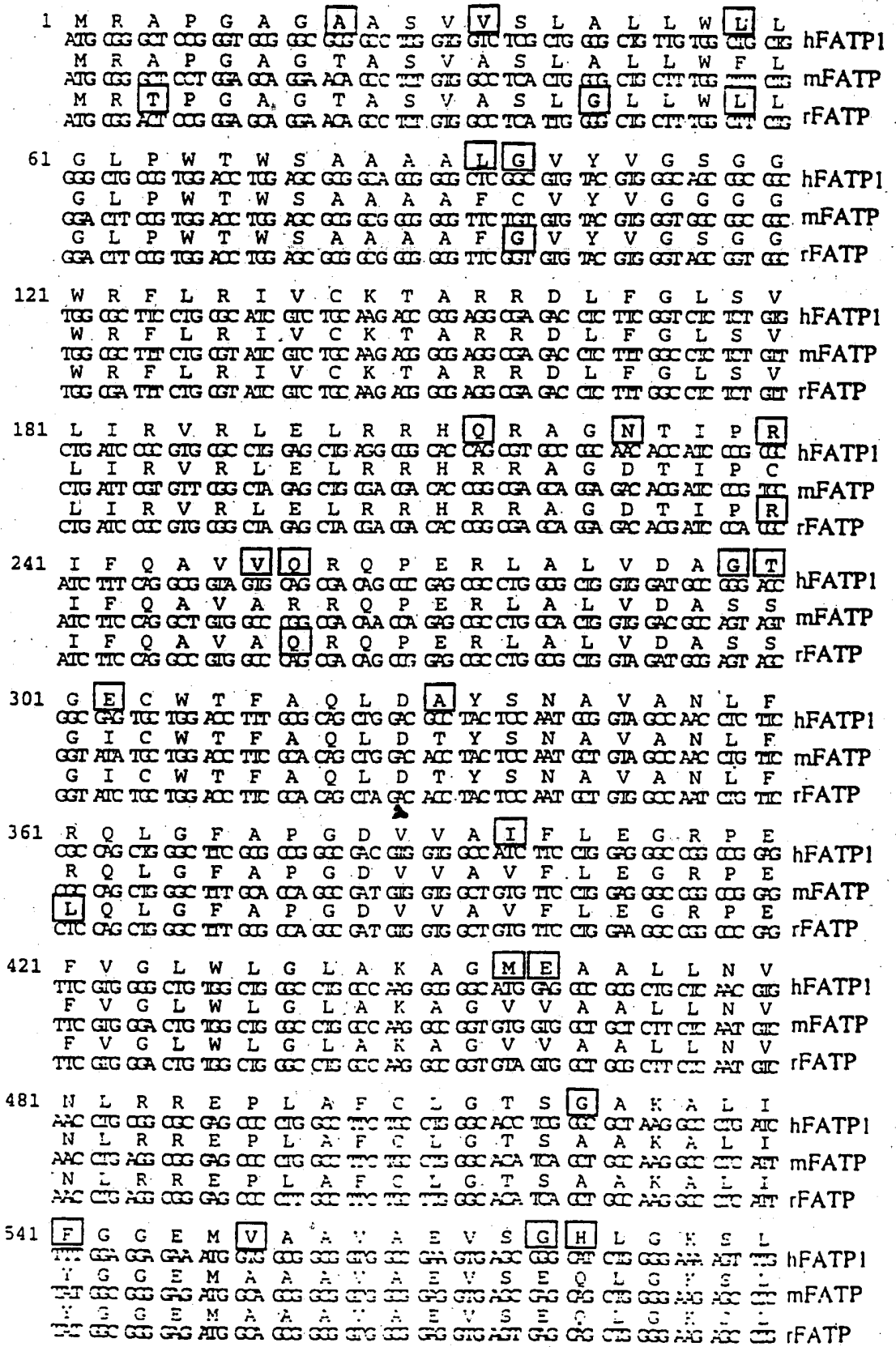

1 M R A P G A G A A S V V S L A L L W L L hFATP1
M R A P G A G T A S V A S L A L L W F L mFATP
M R T P G A G T A S V A S L G L L W L L rFATP
61 G L P W T W S A A A A L G V Y V G S G G hFATP1
G L P W T W S A A A A F C V Y V G G G G mFATP
G L P W T W S A A A A F G V Y V G S G G rFATP
121 W R F L R I V C K T A R R D L F G L S V hFATP1
W R F L R I V C K T A R R D L F G L S V mFATP
W R F L R I V C K T A R R D L F G L S V rFATP
181 L I R V R L E L R R H Q R A G N T I P R hFATP1
L I R V R L E L R R H R R A G D T I P C mFATP
L I R V R L E L R R H R R A G D T I P R rFATP
241 I F Q A V V Q R Q P E R L A L V D A G T hFATP1
I F Q A V A R R Q P E R L A L V D A S S mFATP
I F Q A V A Q R Q P E R L A L V D A S S rFATP
301 G E C W T F A Q L D A Y S N A V A N L F hFATP1
G I C W T F A Q L D T Y S N A V A N L F mFATP
G I C W T F A Q L D T Y S N A V A N L F rFATP
361 R Q L G F A P G D V V A I F L E G R P E hFATP1
R Q L G F A P G D V V A V F L E G R P E mFATP
L Q L G F A P G D V V A V F L E G R P E rFATP
421 F V G L W L G L A K A G M E A A L L N V hFATP1
F V G L W L G L A K A G V V A A L L N V mFATP
F V G L W L G L A K A G V V A A L L N V rFATP
481 N L R R E P L A F C L G T S G A K A L I hFATP1
N L R R E P L A F C L G T S A A K A L I mFATP
N L R R E P L A F C L G T S A A K A L I rFATP
541 F G G E M V A A V A E V S G H L G K S L hFATP1
Y G G E M A A A V A E V S E Q L G Y S L mFATP
Y G G E M A A A V A E V S E Q L G K S L rFATP

FIG. 2A

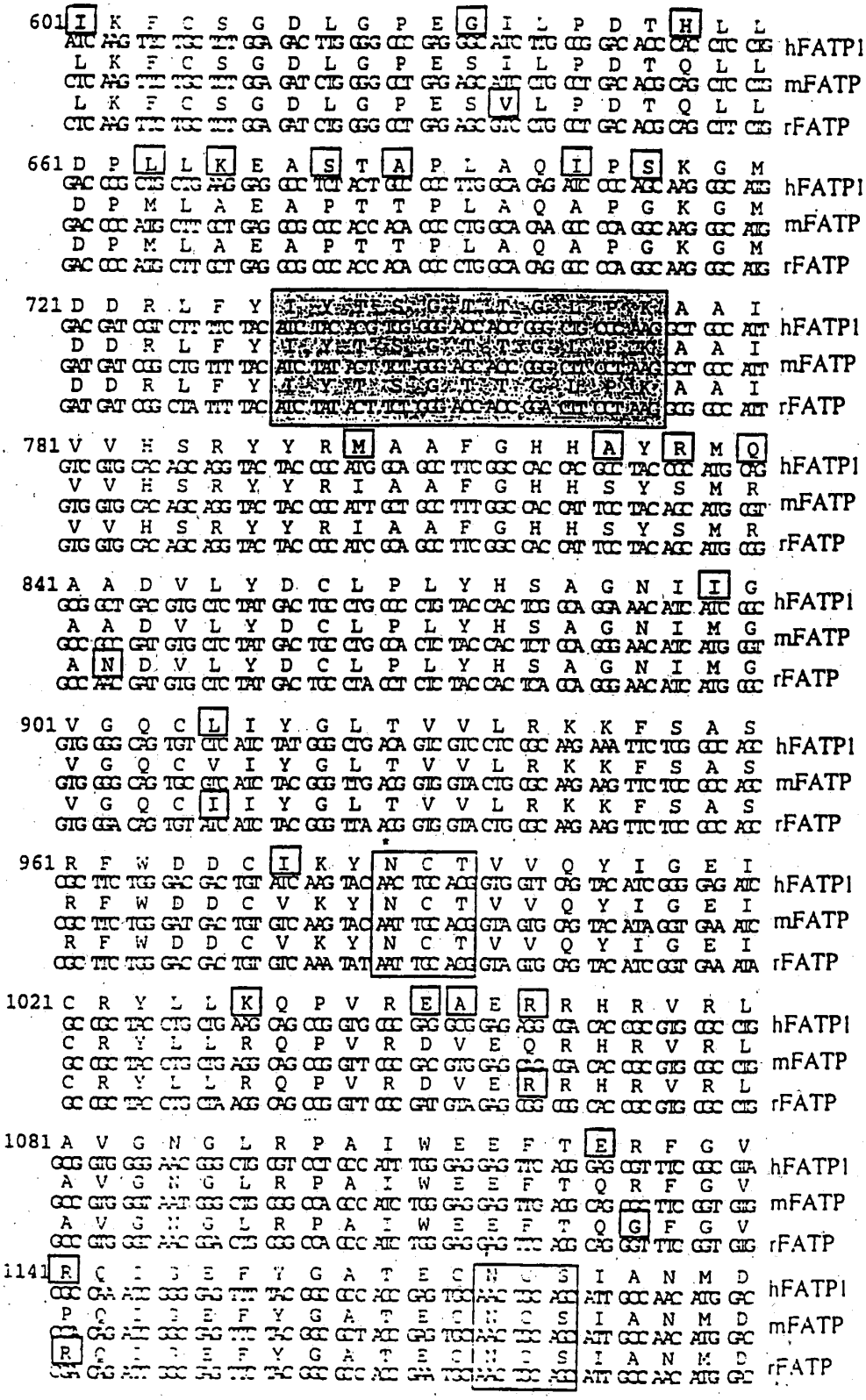


FIG. 2B

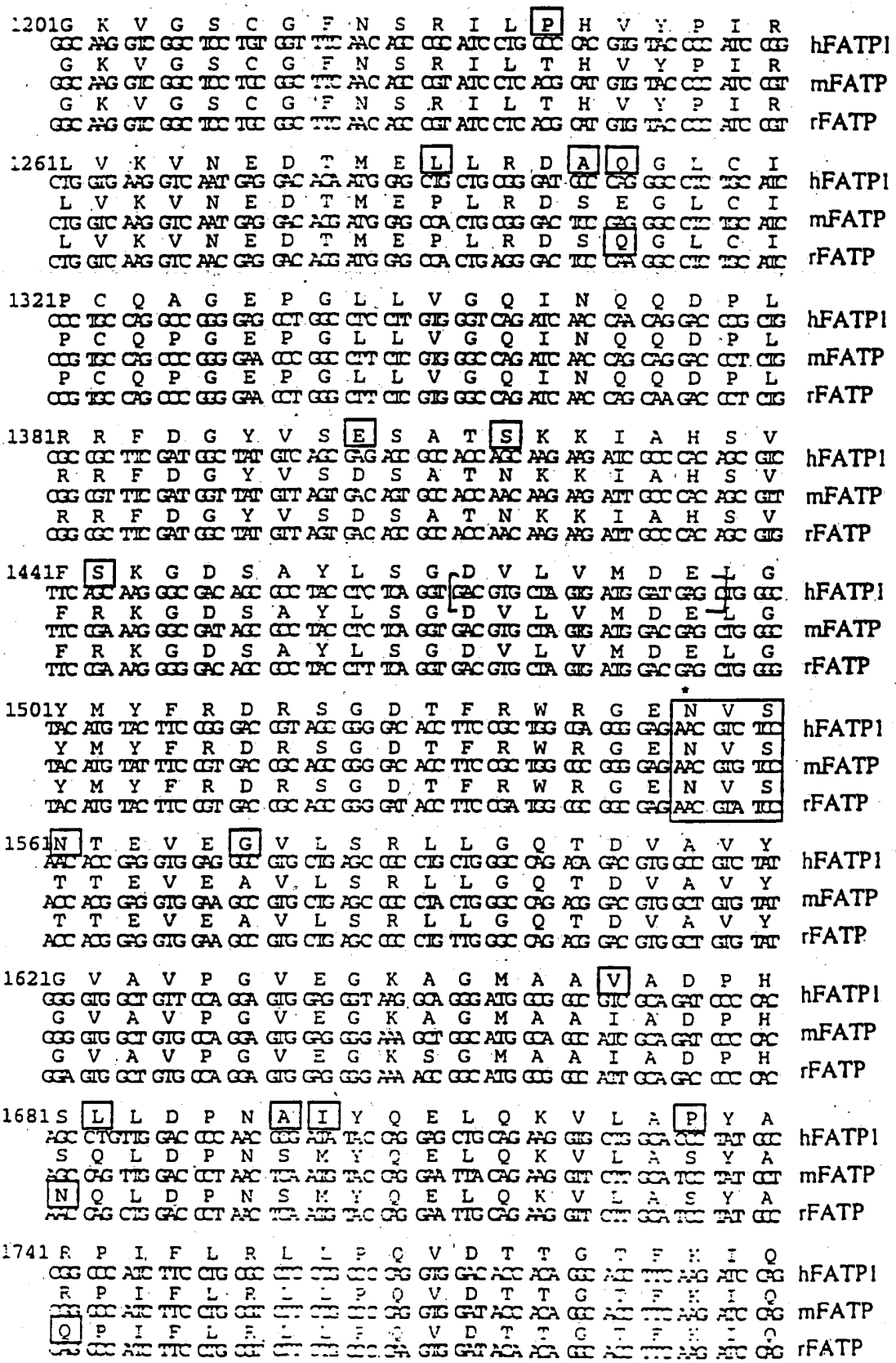

1201 G K V G S C G F N S R I L P H V Y P I R hFATP1
G K V G S C G F N S R I L T H V Y P I R mFATP
G K V G S C G F N S R I L T H V Y P I R rFATP
1261 L V K V N E D T M E L L R D A Q G L C I hFATP1
L V K V N E D T M E P L R D S E G L C I mFATP
L V K V N E D T M E P L R D S Q G L C I rFATP
1321 P C Q A G E P G L L V G Q I N Q Q D P L hFATP1
P C Q P G E P G L L V G Q I N Q Q D P L mFATP
P C Q P G E P G L L V G Q I N Q Q D P L rFATP
1381 R R F D G Y V S E S A T S K K I A H S V hFATP1
R R F D G Y V S D S A T N K K I A H S V mFATP
R R F D G Y V S D S A T N K K I A H S V rFATP
1441 F S K G D S A Y L S G D V L V M D E L G hFATP1
F R K G D S A Y L S G D V L V M D E L G mFATP
F R K G D S A Y L S G D V L V M D E L G rFATP
1501 Y M Y F R D R S G D T F R W R G E N V S hFATP1
Y M Y F R D R S G D T F R W R G E N V S mFATP
Y M Y F R D R S G D T F R W R G E N V S rFATP
1561 N T E V E G V L S R L L G Q T D V A V Y hFATP1
T T E V E A V L S R L L G Q T D V A V Y mFATP
T T E V E A V L S R L L G Q T D V A V Y rFATP
1621 G V A V P G V E G K A G M A A V A D P H hFATP1
G V A V P G V E G K A G M A A I A D P H mFATP
G V A V P G V E G K S G M A A I A D P H rFATP
1681 S L L D P N A I Y Q E L Q K V L A P Y A hFATP1
S Q L D P N S M Y Q E L Q K V L A S Y A mFATP
N Q L D P N S M Y Q E L Q K V L A S Y A rFATP
1741 R P I F L R L L P Q V D T T G T F H I Q hFATP1
R P I F L R L L P Q V D T T G T F H I Q mFATP
Q P I F L R L L P Q V D T T G T F H I Q rFATP

FIG. 2C

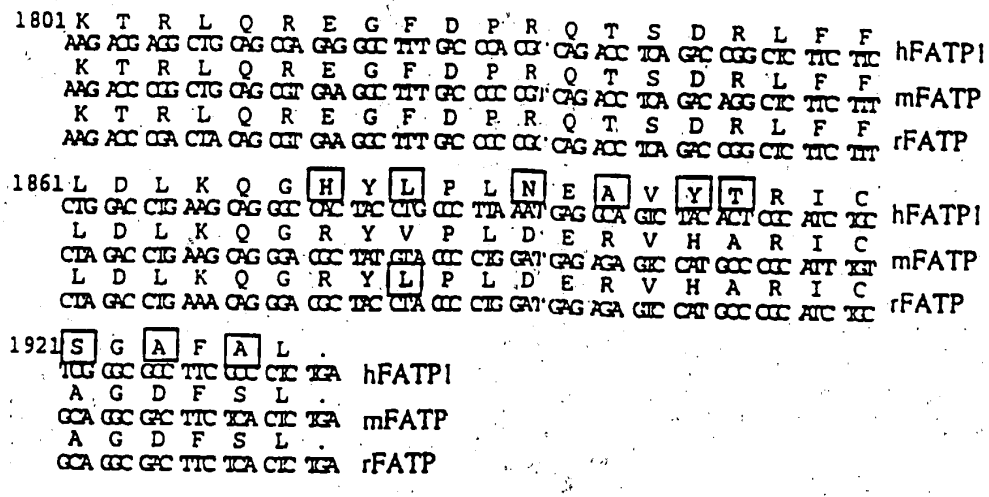

1801 K T R L Q R E G F D P R Q T S D R L F F
AAG ACG AGG CTG CAG CGA GAG GCC TTT GAC CCA CGT CAG ACC TCA GAC CGG CTC TTC TTC hFATP1
K T R L Q R E G F D P R Q T S D R L F F
AAG ACC CGG CTG CAG CGT GAA GCC TTT GAC CCC CGT CAG ACC TCA GAC AGG CTC TTC TTT mFATP
K T R L Q R E G F D P R Q T S D R L F F
AAG ACC CGA CTA CAG CGT GAA GCC TTT GAC CCC CGC CAG ACC TCA GAC CGG CTC TTC TTT rFATP
1861 L D L K Q G H Y L P L N E A V Y T R I C
CTG GAC CTG AAG CAG GGC CAC TAC CTG CCC TTA AAT GAG GCA GTC TAC ACT CGC ATC TGC hFATP1
L D L K Q G R Y V P L D E R V H A R I C
CTA GAC CTG AAG CAG GGA CGC TAT GTA CCC CTG GAT GAG AGA GTC CAT GCC CGC ATT TGT mFATP
L D L K Q G R Y L P L D E R V H A R I C
CTA GAC CTG AAA CAG GGA CGC TAC CTA CCC CTG GAT GAG AGA GTC CAT GCC CGC ATC TGC rFATP
1921 S G A F A L .
TCG GGC GCC TTC GCC CTC TGA hFATP1
A G D F S L .
GCA GGC GAC TTC TCA CTC TGA mFATP
A G D F S L .
GCA GGC GAC TTC TCA CTC TGA rFATP

FIG. 2D

FIG. 3

hsFATP1 cDNA

Start Codon

```
CGGGGAGACG GGACGTGAAG GGATGCGGGC TCCTGGAGCA GGAACAGCCT CTGTGGCCTC 60
ACTGGCGCTG CTTTGGTTTC TGGGACTTCC GTGGACCTGG AGCGCGGCGG CGGCGTTCTG 120
TGTGTACGTG GGTGGCGGCG GCTGGCGCTT TCTGCGTATC GTCTGCAAGA CGGCGAGGCG 180
AGACCTCTTT GGCCTCTCTG TTCTGATTCG TGTTCGGCTA GAGCTGCGAC GACACCGGCG 240
AGCAGGAGAC ACGATCCCGT GCATCTTCCA GGCTGTGGCC CGGCGACAAC CAGAGCGCCT 300
GGCACTGGTG GACGCCAGTA GTGGTATATG CTGGACCTTC GCACAGCTGG ACACCTACTC 360
CAATGCTGTA GCCAACCTGT TCCGCCAGCT GGGCTTTGCA CCAGGCGATG TGGTGGCTGT 420
GTTCCTGGAG GGCCGGCCGG AGTTCGTGGG ACTGTGGCTG GGCCTGGCCA AGGCCGGTGT 480
GGTGGCTGCT CTTCTCAATG TCAACCTGAG GCGGGAGCCC CTGGCCTTCT GCCTGGGCAC 540
ATCAGCTGCC AAGGCCCTCA TTTATGGCGG GGAGATGGCA GCGGCGGTGG CGGAGGTGAG 600
CGAGCAGCTG GGGAAGAGCC TCCTCAAGTT CTGCTCTGGA GATCTGGGGC CTGAGAGCAT 660
CCTGCCTGAC ACGCAGCTCC TGGACCCCAT GCTTGCTGAG GCGCCCACCA CACCCCTGGC 720
ACAAGCCCCA GGCAAGGGCA TGGATGATCG GCTGTTTTAC ATCTATACTT CTGGGACCAC 780
CGGGCTTCCT AAGGCTGCCA TTGTGGTGCA CAGCAGGTAC TACCGCATTG CTGCCTTTGG 840
CCACCATTCC TACAGCATGC GTGCCGCCGA TGTGCTCTAT GACTGCCTGC CACTCTACCA 900
CTCTGCAGGG AACATCATGG GTGTGGGGCA GTGCGTCATC TACGGGTTGA CGGTGGTACT 960
GCGCAAGAAG TTCTCCGCCA GCCGCTTCTG GGATGACTGT GTCAAGTACA ATTGCACGGT 1020
AGTGCAGTAC ATAGGTGAAA TCTGCCGCTA CCTGCTGAGG CAGCCGGTTC GCGACGTGGA 1080
GCAGCGACAC CGCGTGCGCC TGGCCGTGGG TAATGGGCTG CGGCCAGCCA TCTGGGAGGA 1140
GTTCACGCAG CGCTTCGGTG TGCCACAGAT CGGCGAGTTC TACGGCGCTA CCGAGTGCAA 1200
CTGCAGCATT GCCAACATGG ACGGCAAGGT CGGCTCCTGC GGCTTCAACA GCCGTATCCT 1260
CACGCATGTG TACCCCATCC GTCTGGTCAA GGTCAATGAG GACACGATGG AGCCACTGCG 1320
GGACTCCGAG GGCCTCTGCA TCCCGTGCCA GCCCGGGGAA CCCGGCCTTC TCGTGGGCCA 1380
GATCAACCAG CAGGACCCTC TGCGGCGTTT CGATGGTTAT GTTAGTGACA GTGCCACCAA 1440
CAAGAAGATT GCCCACAGCG TTTTCCGAAA GGGCGATAGC GCCTACCTCT CAGGTGACGT 1500
GCTAGTGATG GACGAGCTGG GCTACATGTA TTTCCGTGAC CGCAGCGGGG ACACCTTCCG 1560
CTGGCGCGGG GAGAACGTGT CCACCACGGA GGTGGAAGCC GTGCTGAGCC GCCTACTGGG 1620
CCAGACGGAC GTGGCTGTGT ATGGGGTGGC TGTGCCAGGA GTGGAGGGGA AAGCTGGCAT 1680
GGCAGCCATC GCAGATCCCC ACAGCCAGTT GGACCCTAAC TCAATGTACC AGGAATTACA 1740
GAAGGTTCTT GCATCCTATG CTCGGCCCAT CTTCCTGCGT CTTCTGCCCC AGGTGGATAC 1800
CACAGGCACC TTCAAGATCC AGAAGACCCG GCTGCAGCGT GAAGGCTTTG ACCCCCGTCA 1860
GACCTCAGAC AGGCTCTTCT TTCTAGACCT GAAGCAGGGA CGCTATGTAC CCCTGGATGA 1920
GAGAGTCCAT GCCCGCATTT GTGCAGGCGA CTTCTCACTC TGAAGCTGTT CCTCTACTGG 1980
CCACAAACTC TGGGCGTGGT GGGAGAGGCC AGCTTGAGCC AGACAGCGCT GCCCAGGGGT 2040
GGCCGCCTAG TACACACCCA CCTGGCCGAG CTGTACCTGG CACGGCCCAT CCTGGACTGA 2100
GAAACTGGAA CCTCAGAGGA ACCCGTGCCT CTCTGCTGCC TTGGTGCCCC TGTGTCTGCC 2160
TCCTCTCCCT GCTTTTCAGC CTNTGTCTCC TTCCATCCNT GTCCCTGTNT GGCCTTAACC 2220
CG                                                                2222
```

Stop Codon

hs FATP1

FIG. 4A

```
ATGCGGGCTC CGGGTGCGGG CGCGGCCTCG GTGGTCTCGC TGGCGCTGTT GTGGCTGCTG 60
GGGCTGCCGT GGACCTGGAG CGCGGCAGCG GCGCTCGGCG TGTACGTGGG CAGCGGCGGC 120
TGGCGCTTCC TGCGCATCGT CTGCAAGACC GCGAGGCGAG ACCTCTTGTG AGTGTTGCGG 180
GATCCAGGGC TGGGGGCGGG GCTGAGGGCT CTGGGGGCCC ACGCTGCAGG GCTGGGCTTG 240
CGGGAGGCCT TGGAGGTGGA GAGTGAXXXX XXXXXATACG CTGAGATCTA CTCTCTGCTG 300
TGTAATGCTG CCTGGTCACT GAGAGATCAG CACAAAGTTC ACATCGCCTC ATGAAAGCCT 360
GCTGCCTGGG TCTCAGCGGG AGGCTGAGGC TCCAGAGGCC AGGCGGGGCA GGGCACCAGT 420
GACGCTGTCC CTCCGTCCTC CCTCCCAGCG GTCTCTCTGT GCTGATCCGC GTGCGCCTGG 480
AGCTGCGGGG CCACCAGCGT GCCGGCCACG GTCTCTCTGT GCTGATCCGC GTGCGCCTGG 540
AGCTGCGGCG GCACCAGCGT GCCGGCCACA CCATCCCGCG CATCTTTCAG GCGGTAGTGC 600
AGCGACAGCC CGAGCGCCTG GCGCTGGTGG ATGCCGGGAC CGGCGAGTGC TGGACCTTTG 660
CGCAGCTGGA CGCCTACTCC AATGCGGTAG CCAACCTCTT CCGCCAGCTG GGCTTCGCGC 720
CGGGCGACGT GGTGGCCATC TTCCTGGAGG GCCGGCCGGA GTTCGTGGGG CTGTGGCTGG 780
GCCTGGCCAA GGCGGGCATG GAGGCCGCGC TGCTCAACGT GAACCTGCGG CGCGAGCCCC 840
TGGCCTTCTG CCTGGGCACC TCGGGCGCTA AGGCCCTGAT CTTTGGAGGA GAAATGGTGG 900
CGGGTGAGGC CAGGCGTGGG CATCAGGTGG GCGGGGACCC AGGACTGGCC CCTGGGCGGG 960
CGGGGAGTCT GCTGCGCCCC AGGCCTCGGA AGGCGGCCGC CTGGACGTGG GCATGAGGTG 1020
CACGGTCTGG GTATGCCCCG GGCAGGGAGT TGGTGCATCC CAGGCCTCGG GAGGGGCCTG 1080
TCCGGCGGTG ACCATGACCC ATGTGTTGGG GACCACAGCG GTGGCCGAAG TGAGCGGGCA 1140
TCTGGGGAAA AGTTTGATCA AGTTCTGCTC TGGAGACTTG GGGCCCGAGG GCATCTTGCC 1200
GGACACCCAC CTCCTGGACC CGCTGCTGAA GGAGGCCTCT ACTGCCCCCT TGGCACAGAT 1260
CCCCAGCAAG GGCATGGACG GTGAGTCAAC TTCCAGGACA CCTCTACCCC AATGACTCAG 1320
GCCCCACCCC CTAACACTGT ATCTCCTGCA GATCGTCTTT TCTACATCTA CACGTCGGGG 1380
ACCACCGGGC TGCCCAAGGC TGCCATTGTC GTGCACAGCA GGTGAGGGGC CCACAGGCAT 1440
AATGCCCTCA GCCGCTGAGA GTGACCCAGG CATCTTGCCA GCCTGACCTG CCCCTCAGCT 1500
CCTGTGGGCA TCTCCATGTT ACCCTGGGGA CAGAGAGGGC AGCTGGTGTT TCCTGAGCAC 1560
TTGCTCTGTG TCCAGACCAG GGCCAAGCCC TGGACGTGTA AACTCATTGC AAGGGTCATA 1620
ACTGCTTGGG GCTGGACAAA GGCATCACAC CATTTTCACA CCATCTTGGG ACCXXXXXXX 1680
XXXXXXXXGG GGATTTAGGT CCAGCCTCTG CCTCCGGCTC CCCCTCCCCC TGCAGGTACT 1740
ACCGCATGGC AGCCTTCGGC CACCACGCCT ACCGCATGCA GGCGGCTGAC GTGCTCTATG 1800
ACTGCCTGCC CCTGTACCAC TCGGCAGGTA CTACGGCCTG GGTAGGGAAT GGTGGGTGGG 1860
GGCGGGGGAC CCCTTACCAA GGCCACCCTC TGCAGGAAAC ATCATCGGCG TGGGGCAGTG 1920
TCTCATCTAT GGGCTGACAG TCGTCCTCCG CAAGAAATTC TCGGCCAGCC GCTTCTGGGA 1980
CGACTGCATC AAGTACAACT GCACGCCTTG GCNNTCCGAA AGTGCTGAGA GNACAGGCGT 2040
GAGCTTTGTG CCCGGCCAGG GCCCAGCCCT TTTATCGGTG CAGCTGCTGG CATCCCCTGC 2100
TGCAAGCTTC CAGCTTCCTT ATTTGTGTTT GCAGCCCAAT CTTCCAGGCT GTTCTTTGTT 2160
AGAAAATAAG TGATTTCTTG GGCTGCTTTT TGTTAGAANN GAAGTTCTAC CGAGGGTNNC 2220
NTGTTTCCAA AAAAAAAAAA TAGGNAAATT NGATTAGATA CTNACTNGCA TAATANGNGA 2280
CNGNGTTGGG NNCTTCCANA GATTTGCGCC CCTATGCGGA TGGCACATCT GGTCCTCACC 2340
GGTTTTTCAC GGTCTATTTA GATCAGATAC AGCCTCCCTG NTAGCTTGTN TGTAAAAATC 2400
CTTGCCTTTT TTTTTTTTTC TGAGGCGGAG TCTCACTCTT GTCCCCAGGC TGGAGTACAA 2460
TAGAGCGATC TTGGCTTACT GCAACCTCTG CCTCTCGGGT TCAAGAGATT CTCCTGCCTC 2520
AGCCTCCCAA GTAGCTGGGA TTACAGGCAC ATGCCACCAG GTCTGGCAAA ATTTTGTTGT 2580
ATTTTTGTAG AGATGAGGTA TTACTACATT GGNTAGGCTG GTCTCGAACT TCTGACCTGA 2640
NGTGATCTGC CCACCTNGGN CCCCCCAAAG TGCTGGGANN ACAGGCATGA GCCACGGNGC 2700
CCAGCCATGT GCCCANTTTT TTTTTTTTTT TTGAGATGGA GTCTNGGTNT TGTCGCCCCA 2760
GGCTGGAGTG CCATTGGGCG AANTTGGNCC AATTTNAAGC TCCGCCTCCC GGGTNTACGC 2820
CATTCTCNTG CCTNAGCCTC CCGAGTAGCT GGGATTACAG GAGTGTGCCA CCACGNCTGG 2880
```

FIG. 4B

NTAATTTTTT GNATTTTTTA GTAGAGACGG GGTTTNACCA TGTTAGCCAG GATGGTCTCG 2940
ATCTCCTGAC NTNGTGATCC ACCTGCTTTG NCNTCCCAAA GTGCTGGGAT NACAGGCTTG 3000
AGCCACCCCG CCTGGCCGTG CCCATTCTTA AGCCAGTCAT ATTGGCNTGA GGAGGGGCTG 3060
GGGTGATTTN CCAGGTTNTG TCGGTAGAGA TNTTGTTGGG TGAACCANAC CCTAAGGCAG 3120
TGGCCCAATT NTGNGAATGG GGGNGGGGAC CTTCCCCCCC TNNGACCAGG TGTTTGTTCT 3180
GCAGCCAGTG ATCTTGGGTG CATGCATTTC TTNTCTGGGC TTCAGTGCTT CATCTCCAAG 3240
NTCAGCTTCA GAGCATGGCG GTGGTGTCTG CTGATCATAT TGGTTTAGTC ATCNGCNNNT 3300
TNGTCAGNAC NNTCTTCTGG GGGANNAAGG GGGNNCAGGG GTGCTCTCAG TGACGTTTGA 3360
GCTGAGACTG AAGAGTTCAG GCAAANGCAT CTGGGGGAAG ACTGCTTCAG GCAAAAGGCT 3420
AGCATGTGCA AAGTCCCTGA GGAGGCAGAG TGTTCAGCGG AGATGGAGGG AGAGGAGGCA 3480
GCAGGGGCGG CAGGGCCAGA TCCAGTTGGC CACAGGTAGA AATGTGGGTT TCATCCTGTG 3540
TGTGAGCGGG AAGCCCCGAG CACANTTGCA GCTGNTATGA TCCCATCTGG TGGGTAATCC 3600
CCTGGCANGC NANGGNTTCA TTCCCATTNT GGGGGCCGGG ATGCCTGGGT TCAAATCAAA 3660
TTCTGCCACT TCCCAGCTCN GTGATCTCGT TCCAGTTCCT TACCATCTGT GAGCCTCGGT 3720
TTCTCCATCT GTAAAGTGGC GGGGGGGGAG TCATTTCCGA AGCCCAACTG GAGATGAGAA 3780
NTNTTAAAGG CACCGTGGGG CACCTAGTGG ATGACCCCAG GAAGNTCTTG CGGGGCTTGA 3840
GAATGTGGCA GGGAGGGAAG ATGTCCTGCA GGGGGAGCCC ATGAGCAGGT GGCTCCGTGG 3900
GCACCTGGGG NTCAGTCCAG ACGCAGCGCA GCCTAGTGGG TGCACCTGGG AGTGGTCTGC 3960
CCAGGGGTGA CGCTCCGGCC AGCCCAGGCT CAGCCAGGGG GCTCTGGGTG ACAGTGTAGG 4020
CTCCAGGCTG AGNGTTGCCT TTNGGGGTGC CAGNTCTCCT GCTAGTCCTG CCCTGTGTGC 4080
CTGCTGAGGC TCAGACAGGG AGTGGTATCT CCAGGAAGCT GCTTGGCAGA ATCGAGGGAC 4140
CACGAAGGGG TGCGGGTACG GGGAGGAGGA AAGACACTGC ATCTGCTTGG ATAGAAGTTT 4200
GCATCCGGCC AGGCACAAGT GGCTCACACC TGGAATCTCA CACTTTGGGA GGCTGAGGCA 4260
GGAGGATTGC TTGAGGCCAG GAGTTCAAGA CCAGCCTGGG CAACAGAGCG AGATCCCCCA 4320
TCTGTACAAA AAAATCTGTA GTCCCAGCTA CTCGGGAGGC TAAGGCTGGA GGATCACTTG 4380
AAGGCACCCC GTTAGAGGCT GCAGTGAGCT GTGATCATGC AACTGCATNC AGCTNGGGGG 4440
GGGCAGGGGG AGACCCCCCC CCNAAAAACA ACAAAAAAAG CTGCATCNTA GACCCTTTGC 4500
AAGAGACTGA ACGAGTCCTA GGAGTCAATG TGGTCCCTAA TGGAGTGTGG ANGATTCTGC 4560
AGCCATCATC ATCCTTAGGC TGTTCCGCTC ATAGGATTAG CTCCCTGGGT GGGGCGGTCT 4620
CGGGGTNTCT ACCTCTGATC CGGGCTCCCC ACCGCCTGCC GGTCCCATCA CCCACTTCCT 4680
CACCCCGTCC CCCAGGTGGT TCAGTACATC GGGGAGATCT GCCGCTACCT GCTGAAGCAG 4740
CCGGTGCGCG AGGCGGAGAG GCGACACCGC GTGCGCCTGG CGGTGGGGAA CGGGCTGCGT 4800
CCTGCCATCT GGGAGGAGTT CACGGAGCGC TTCGGCGTAC GCCAAATCGG GGAGTTCTAC 4860
GGCGCCACCG AGTGCAACTG CAGCATTGCC AACATGGACG GCAAGGTGCA CACCGGCAGG 4920
CCCCGGGGCA GGTCTCGGAG TTCAGGGAAG ACACTTGTCT CCTCTTCCTG GGCCCTGGAT 4980
ACATAAAACA GCCTGGACTG GCGCGGAAGG CTCGCAAGGC GCACGCAGGG CGGTGTAGGA 5040
GATCTGGACT CCGTGCACAC AGAACTCTGA GXXXXXXXXX XXXXXXXGAC TAGCTGTAGG 5100
TTCACACAGA GGATCTACTT CCTGCCAGAC TGAGGTTTGT GGATCAGGAA GTGGCACCAG 5160
CCAGAGGTCT TCTCTTGACT GAATGCAGGC TGGGAAGGTG GGAGGAGGGG GCCTGAGTTG 5220
GAGGCGACGC TTACTACCCT GCTTTTGCAG ATCAGCCAAG GCAGGCAAAG TGTTATGAGA 5280
AGGACCCCGC ATATCCCCGG CTTTCCTACT CAGTTCACCC CATTCCAGGT CGGCTCCTGT 5340
GGTTTCAACA GCCGCATCCT GCCCCACGTG TACCCCATCC GGCTGGTGAA GGTCAATGAG 5400
GACACAATGG AGCTGCTGCG GGATGCCCAG GGCCTCTGCA TCCCCTGCCA GGCCGGGGTG 5460
AGCAGGGCCC CCGCATGGTC CCCACCCGGA GCAGGGGTCC CCACACCCTG CCTGCCTAGC 5520
GCAGCCTGAA CATGGCCTTC TCCCTAGGGG AGCCTGGCCT CCTTGTGGGT CAGATCAACC 5580
AACAGGACCC GCTGCGCCGC TTCGATGGCT ATGTCAGCGA GAGCGCCACC AGCAAGAAGA 5640
TCGCCCACAG CGTCTTCAGC AAGGGCGACA GCGCCTACCT CTCAGGTGCG CAGCTGCTAG 5700
GCCCCGGTGA CTGGCTGTGC GGATGGGGAT CCGCCACCCA TCTGCCCCTC TCCCCTCTGC 5760
CAGGTGACGT GCTAGTGATG GATGAGCTGG GCTACATGTA CTTCCGGGAC CGTAGCGGGG 5820

FIG. 4C

```
ACACCTTCCG CTGGCGAGGG GAGAACGTCT CCACCACCGA GGTGGAGGGC GTGCTGAGCC 5880
GCCTGCTGGG CCAGACAGAC GTGGCCGTCT ATGGGGTGGC TGTTCCAGGC AAGCTGGGGT 5940
TGCAGGGGGT GGTCCTGAGG CATGGTCCTG AGGGAGCTCA GCCAAAAGGG GCTTAGAGGT 6000
ACACATGCCT TTGGCAGTGC ACAACCTGGA CAACTGCTCA TGGCAGCCCA GGAGGAAGCA 6060
CTGGATCTGG AGCCAGTTCA CCTGGGTGAT GTTGAGCCTC AGTTTTGTCA TCAGAAAATG 6120
GGATCATGAA AGCCCACCTG TATTAGGGCT TCAATGAGCC AAGCAGGAGC TCCCCAAAAT 6180
GTGTGGCTGC TTCCATAAAT GTCATCCCAG GTTGGGAGAG ACTGGAGATT ACAGACCTGC 6240
TACTGCTTGA CAGTGTATCT GGTCCTGCTG GTGAGGATGA GAGGCGGGGT GTCCTCAGCT 6300
CCTCTGCCTC CAGGAGTGGA GGGTAAGGCA GGGATGGCGG CCGTCGCAGA CCCCCACAGC 6360
CTGCTGGACC CCAACGCCAT ATACCAGGAG CTGCAGAAGG TGCTGGCACC CTATGCCCGG 6420
CCCATCTTCC TGCGCCTCCT GCCCCAGGTG GACACCACAG GTGCGAGTCT CCCCCACTCC 6480
AACCTCTCTC TTCATCCATC AGTGTGTCTG TTGATTCGAG GGATATTGAG TTGAGGCCTC 6540
CAGAAGCCAC CTGCTCAGCC CTTATCTGCC CCCCATCCCC ACTATAGGCA CCTTCAAGAT 6600
CCAGAAGACG AGGCTGCAGC GAGAGGGCTT TGACCCACGC CAGACCTCAG ACCGGCTCTT 6660
CTTCCTGGAC CTGAAGCAGG GCCACTACCT GCCCTTAAAT GAGGCAGTCT ACACTCGCAT 6720
CTGCTCGGGC GCCTTCGCCC TCTGA                  6745
```

MKAPGAGAASVVSLALLWLLGLPWTWSAAAALGVYVGSGGWRFLRIVCKTARRDLFGLSVLIRVRLELRRHQRAGHTIPRIFQAVVQRQPERLALVDAGT 100

GECWTTFAQLDAYSNAVANLFRQLGFAPGDVVAIFLEGRPEFVGLWLGLAKAGMEAALLNVNLRREPLAFCLGTSGAKALIFGGEMVAAVAEVSGHLGKSL 200

IKFCSGDLGPEGILPDTHLLDPLLKEASTAPLAQIPSKGMDDRLFYIYTSGTTGLPKAAIVVHSRYYRMAAFGHHAYRMQAADVLYDCLPLYHISAGNIIG 300

VGQCLIYGLTVVLRKKFSASRFWDDCIKYNCTVVQYIGEICRYLLKQPVREAERRHRVRLAVGNGLRPAIWEEFTERFGVRQIGEFYGATECNCSIANMD 400

GKVGFGSIGFLSPIVKVNEDTMELLRDAQGLCIPCQAGEPGLLVGQINQQDPLRRFDGYVSESATSKKIAHSVFSKGDSAYLSGDVLVMDELG 500

YMYFVDRMGDTFRWKGENVSTNEVEGVLSRLLGQTDVAVYGVAVPGVEGKAGMAAVADPHSLLDPNAIYQEIQKVLAPYARPIFLRLLPQVDITGTFKIQ 600

KTELQKEGFDPRQTSDPLFILDLKQGHYLPLNEAVYTRICSGAFAL 647

FIG. 5

murine FATP

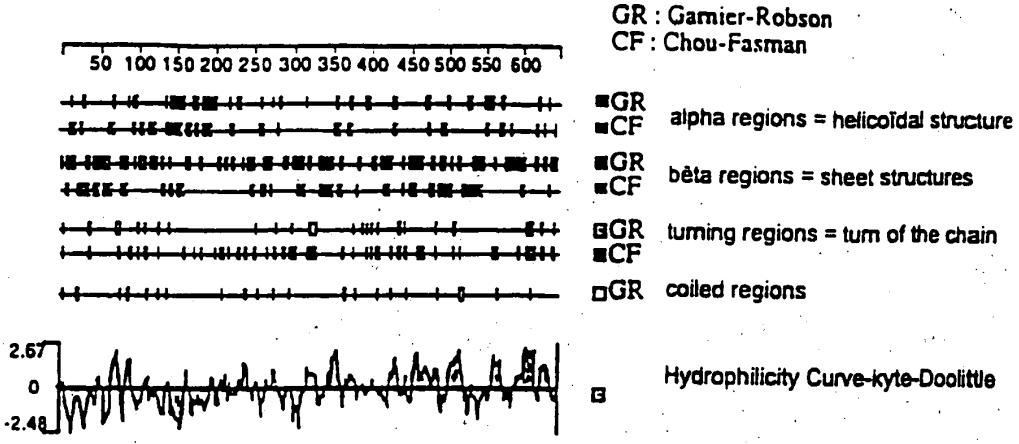


FIGURE 6A

human FATP

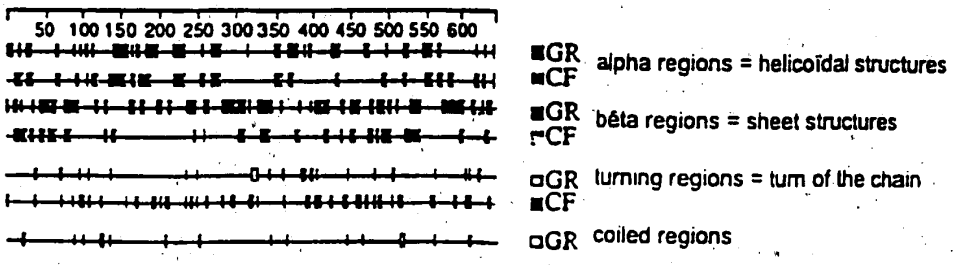


FIG. 6B

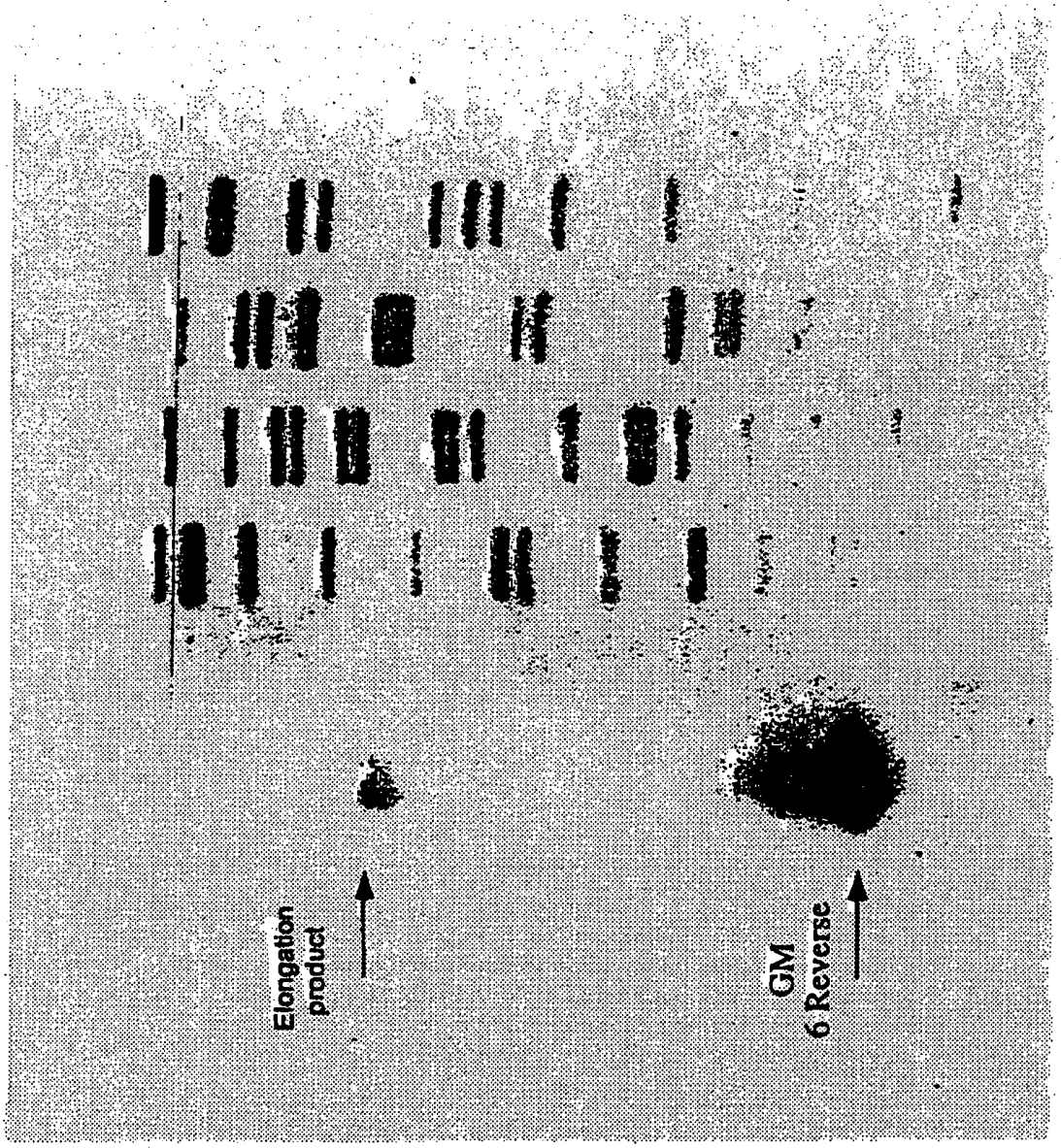


FIG. 7

FIG. 8A

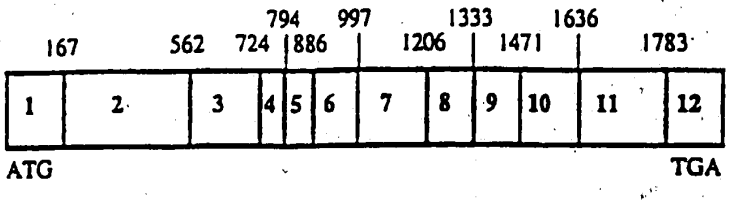


FIG. 8B

Intron 1
GGCGAGACCTCTT--- ? ---CGGTCTCTCTGT
Intron 2
AAATGGTGGCGGgtgaggcc---215---gaccacagCGGTGGCCGAAG
Intron 3
AGGGCATGGACGgtgagtca-----131-----tcctgcagATCGTCTTTTCT
Intron 4
CGTGCACAGCAGgtgagggg--1500--cccctgcagGTACTACCGCAT
Intron 5
ACCACTCGGCAGgtactacg-----68-----ctctgcagGAAACATCATCG
Intron 6
TACAACTGCACG ------->3kb-----cgtcccccacGTCGGCTCCTGT
Intron 7
TGGACGGCAAGgtgcacacc----3000----cattccagGTCGGCTCCTGT
Intron 8
CCCTGCCAGGCCgtgagcag---2900---ctccctagGGGAGCCTGGCC
Intron 9
CCTACCTCTCAGgtgcgcag-----90-----tctgccagGTGACGTGCTAG
Intron 10
TGGCTGTTCCAGgtcaagct----389----gcctccagGAGTGGAGGGTA
Intron 11
TGGACACCACAGgtgcgagt----2800---cactatagGCACCTTCAAGA

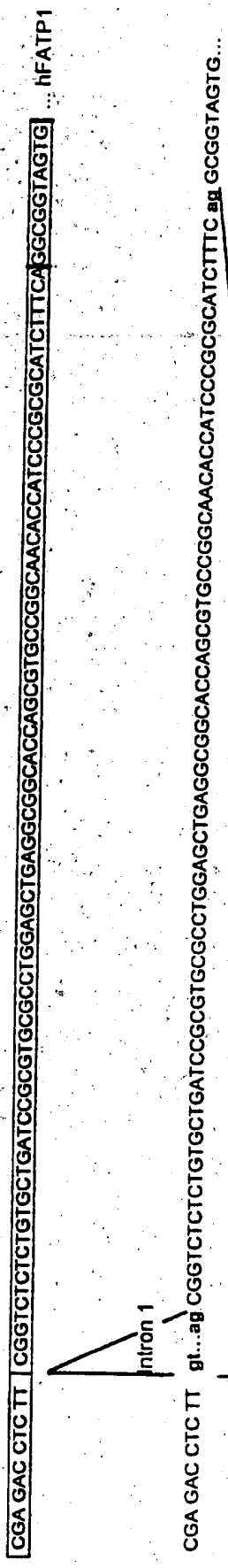

CGA GAC CTC TT
CGGTCTCTCTGTGCTGATCCGCGTGCGCCTGGAGCTGAGGCGGCACCAGCGTGCCGGCAACACCATCCCGCGCATCTTTCAGGCGGTAGTG
...hFATP1
Intron 1
CGA GAC CTC TT gt...ag CGGTCTCTCTGTGCTGATCCGCGTGCGCCTGGAGCTGAGGCGGCACCAGCGTGCCGGCAACACCATCCCGCGCATCTTTC ag GCGGTAGTG...
CGA GAC CTC TT GCGGTAGTGC
...hFATP2

FIG. 8C

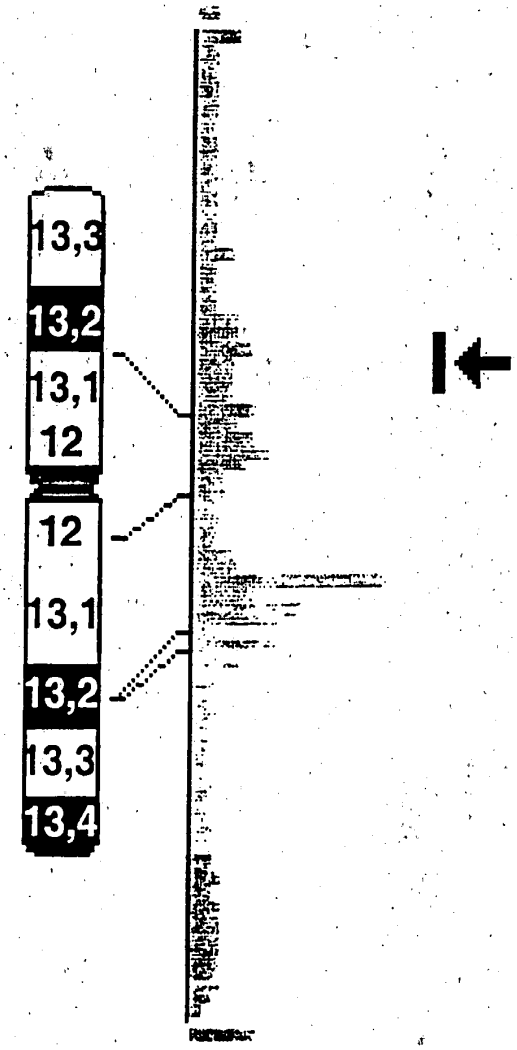


FIG. 9

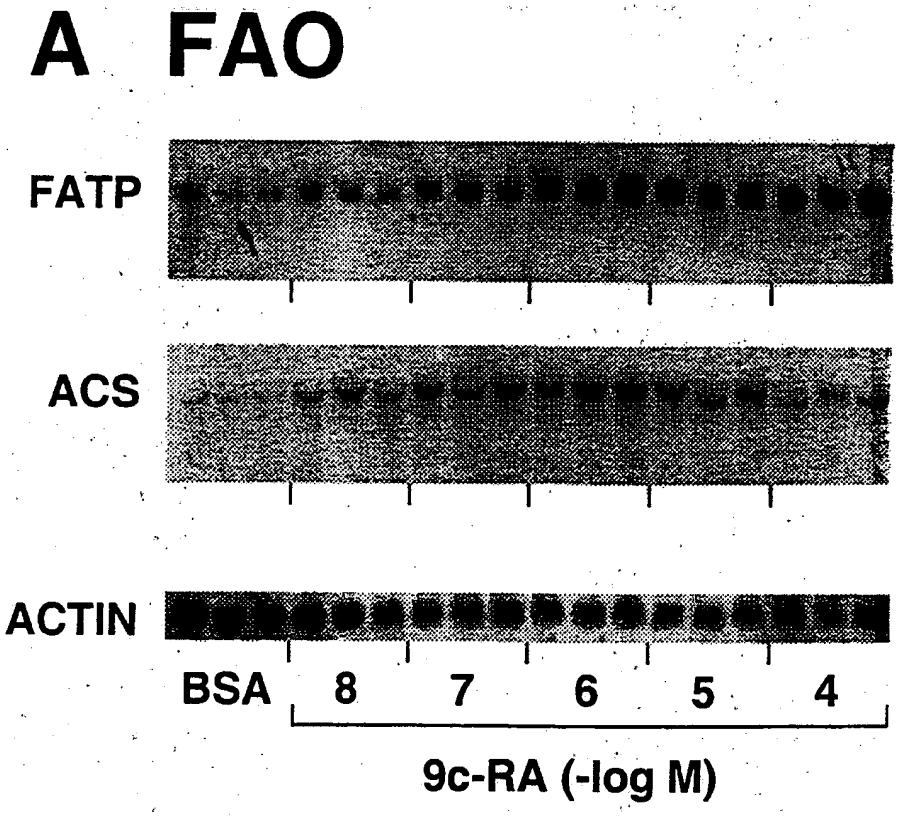


FIG. 10A

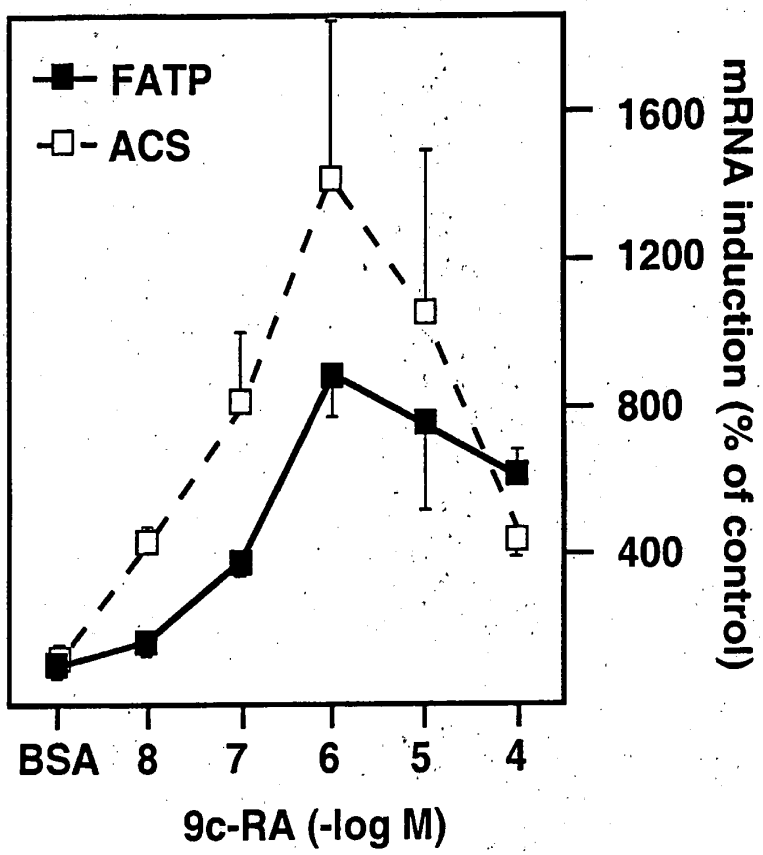

FATP
ACS
1600
1200
800
400
mRNA induction (% of control)
BSA
8
7
6
5
4
9c-RA (-log M)

FIG. 10B

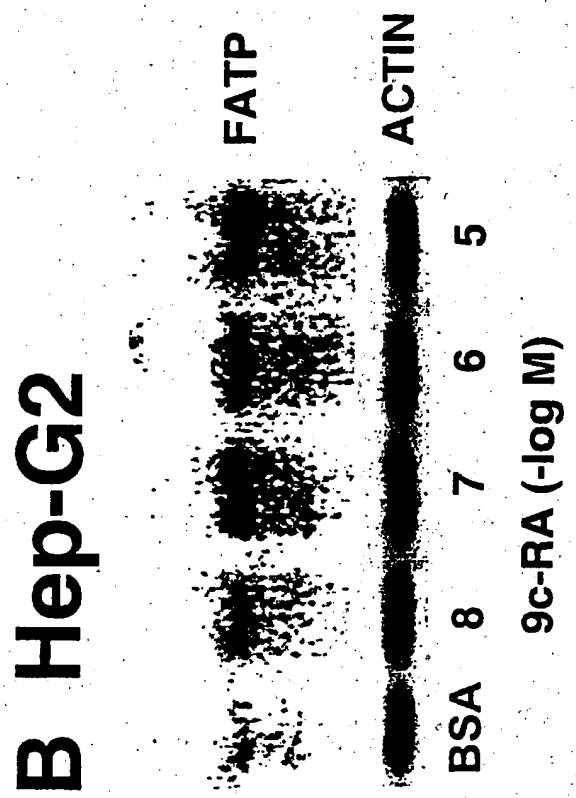


FIG. 10C

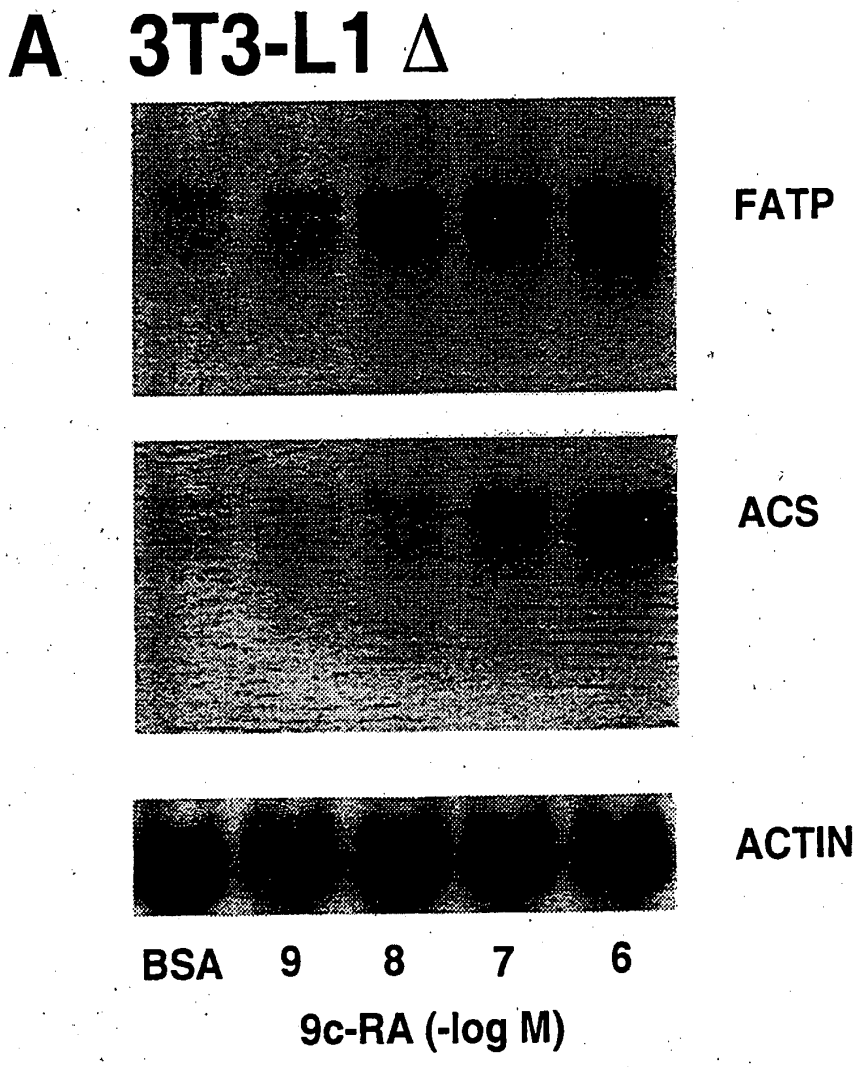


FIG. 11A

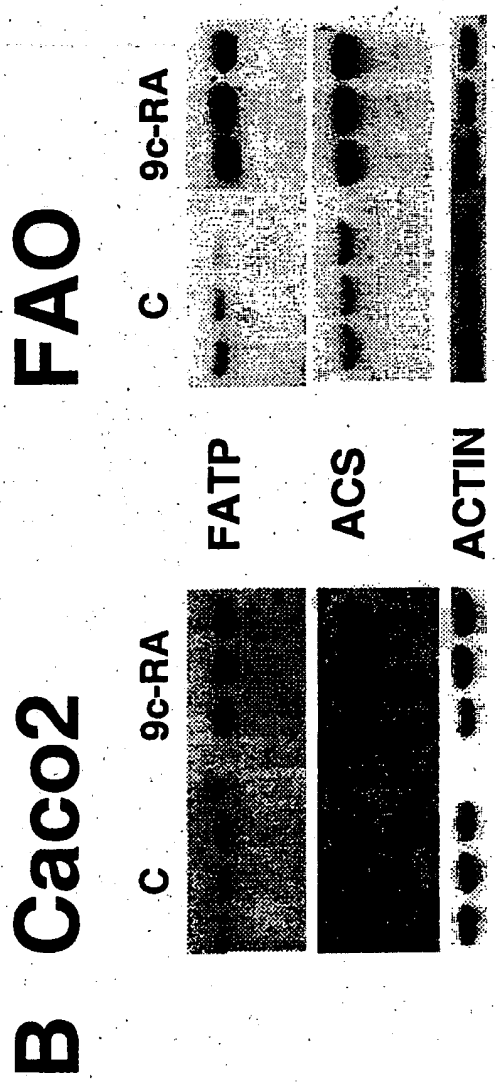
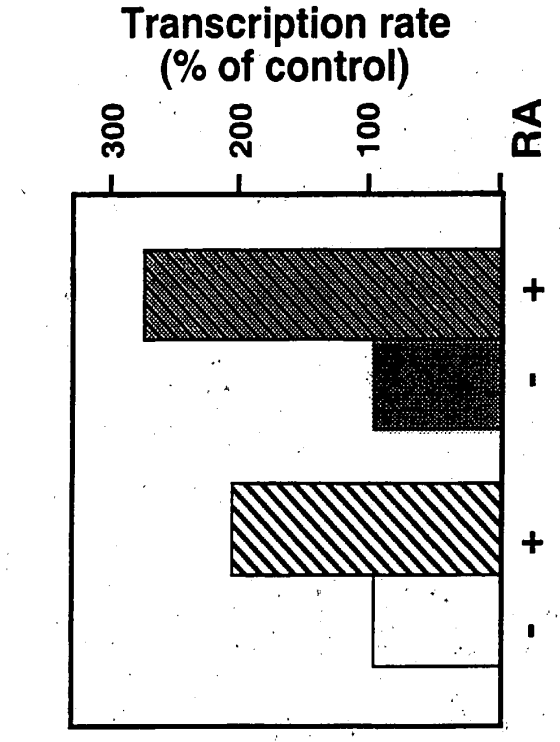


FIG. 11B


Transcription rate
(% of control)
300
200
100
RA
+
-
+
-

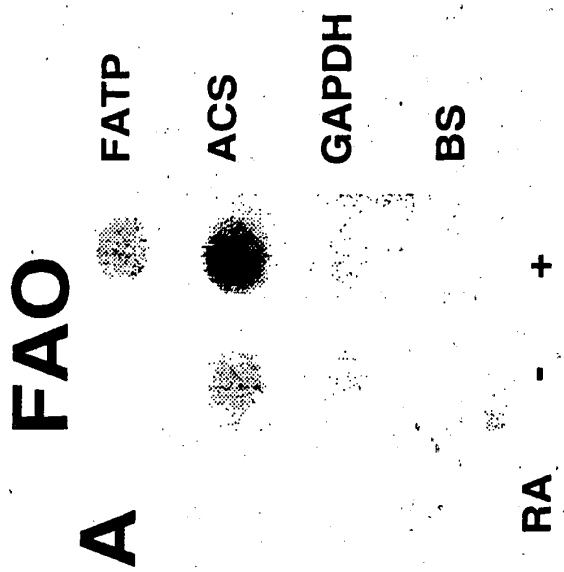

FAO
A
FATP
ACS
GAPDH
BS
+
-
RA

FIG. 12A

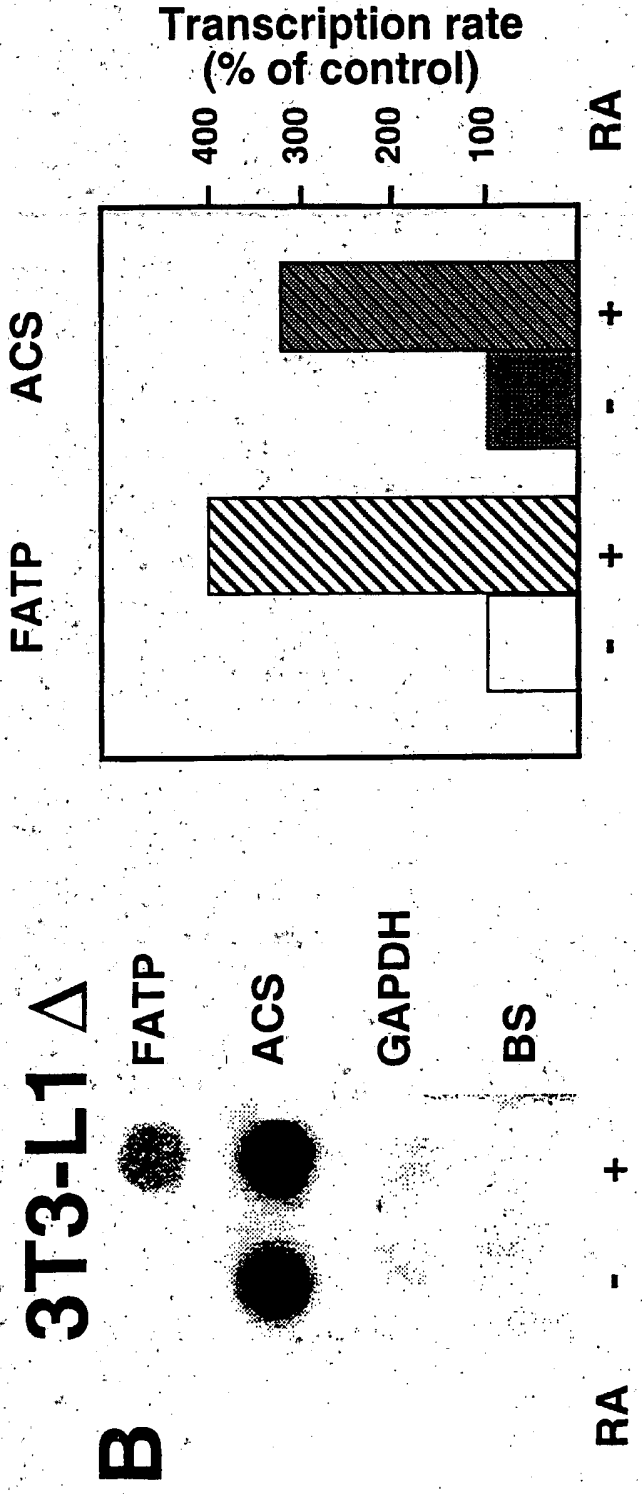

Transcription rate
(% of control)
400
300
200
100
RA
ACS
FATP
+
-
+
-
B
3T3-L1 Δ
FATP
ACS
GAPDH
BS
RA
-
+

FIG. 12B

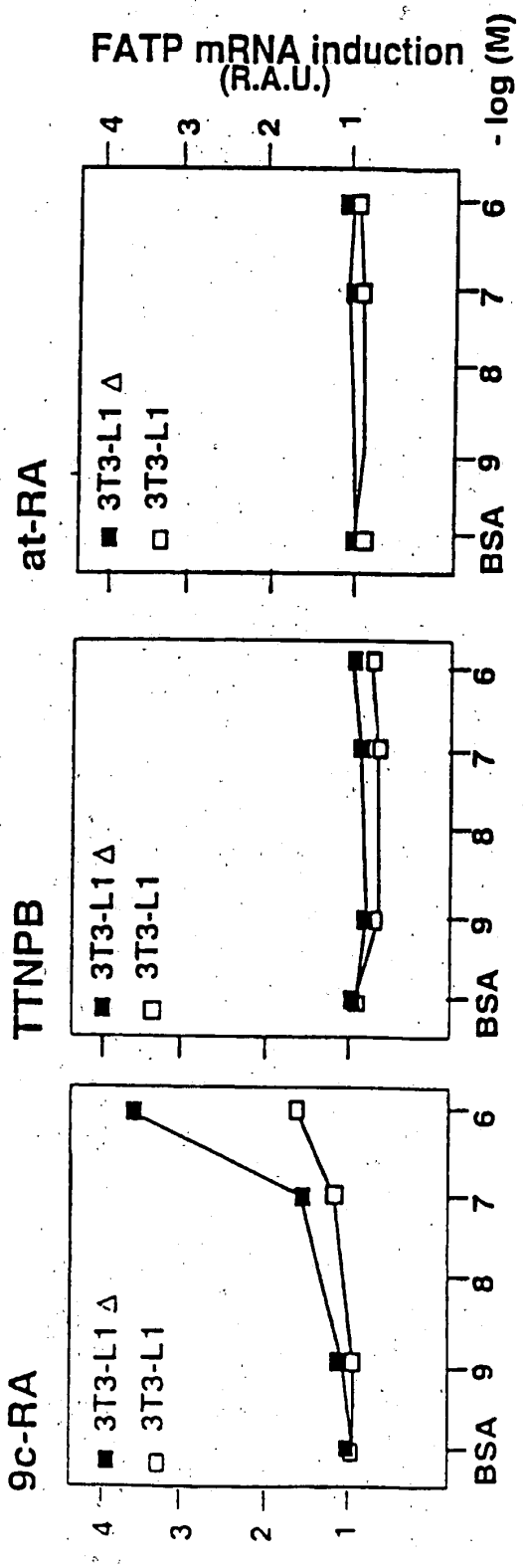

FATP mRNA induction
(R.A.U.)
- log (M)
at-RA
TTNPB
9c-RA
3T3-L1 Δ
3T3-L1
BSA

FIG. 13

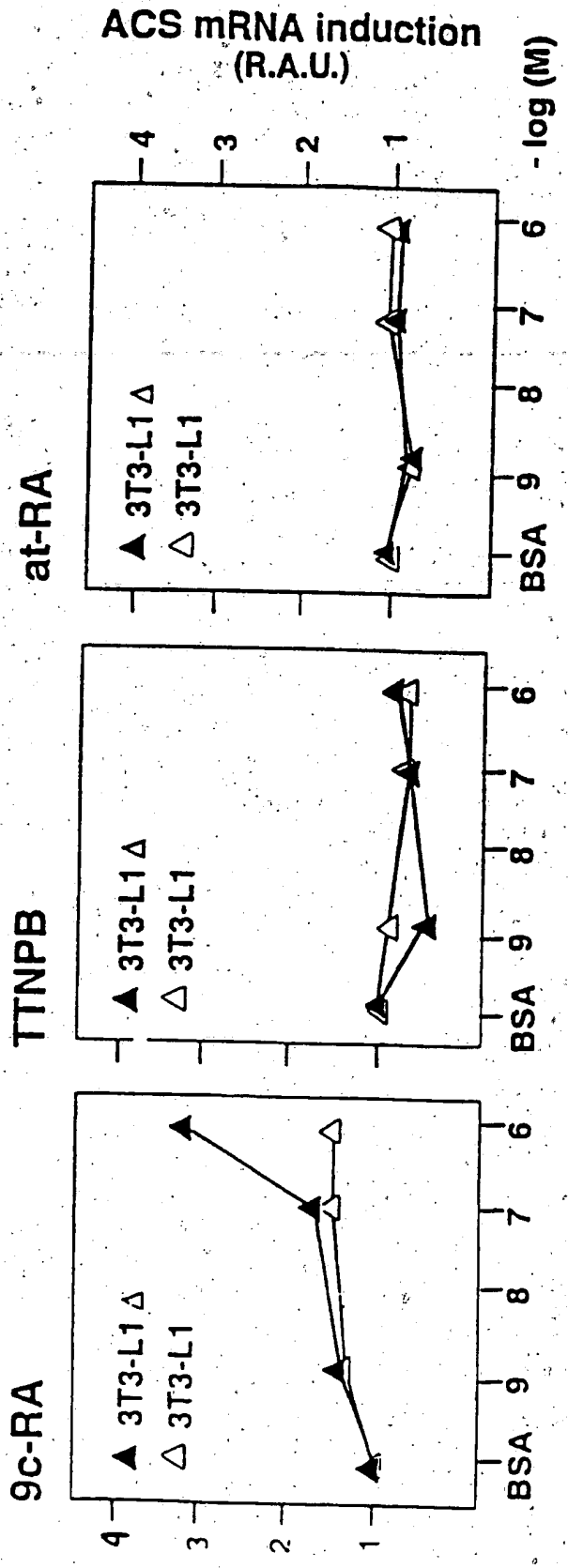

ACS mRNA induction
(R.A.U.)
- log (M)
at-RA
TTNPB
9c-RA
▲ 3T3-L1 Δ
△ 3T3-L1
BSA

FIG. 14

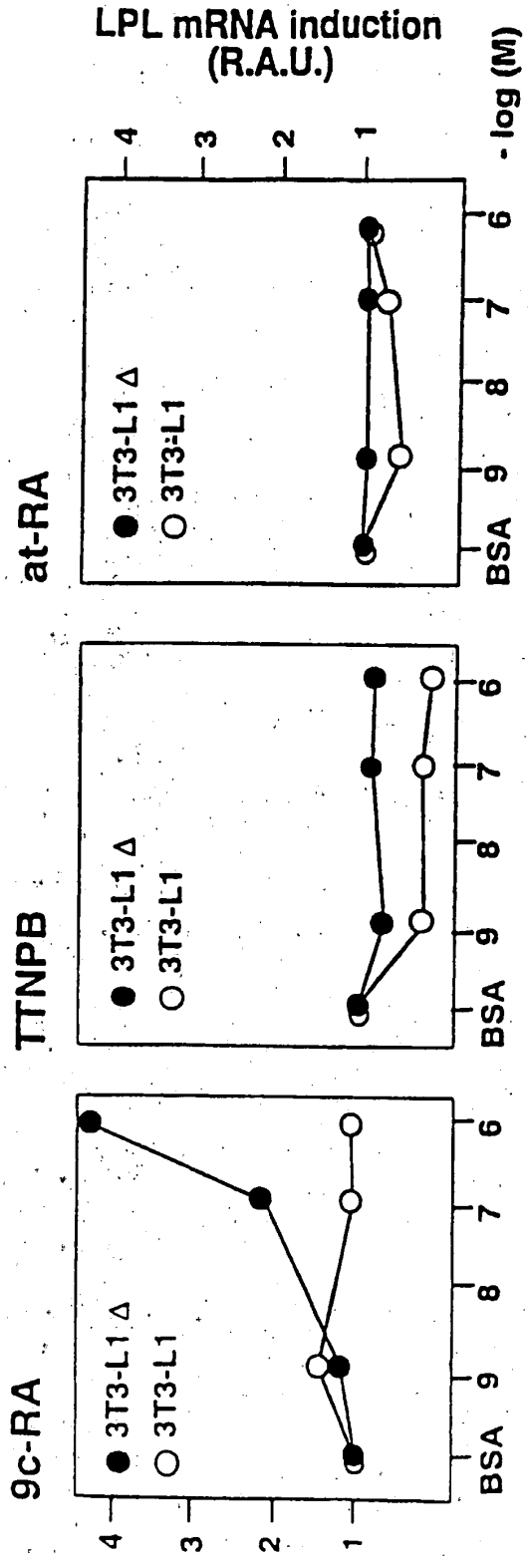

LPL mRNA induction
(R.A.U.)
- log (M)
at-RA
TTNPB
9c-RA
3T3-L1 Δ
3T3-L1
BSA

FIG. 15

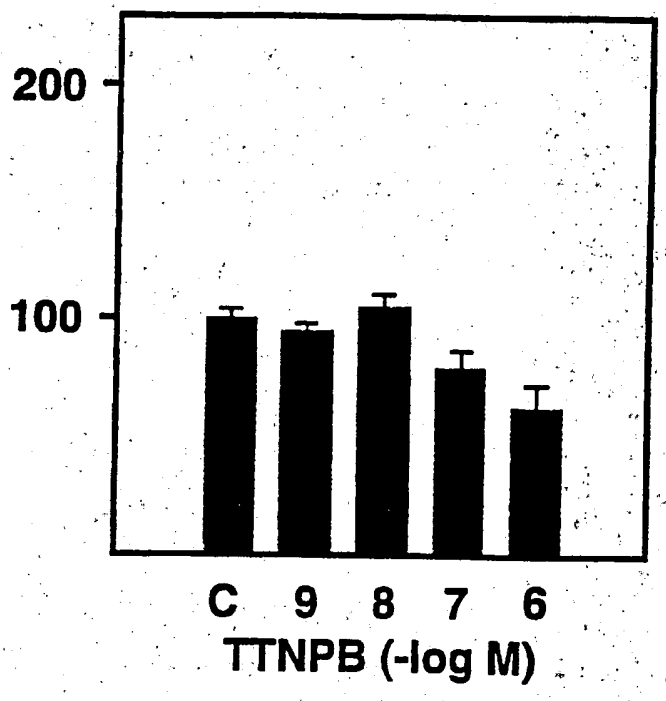


FIG. 16

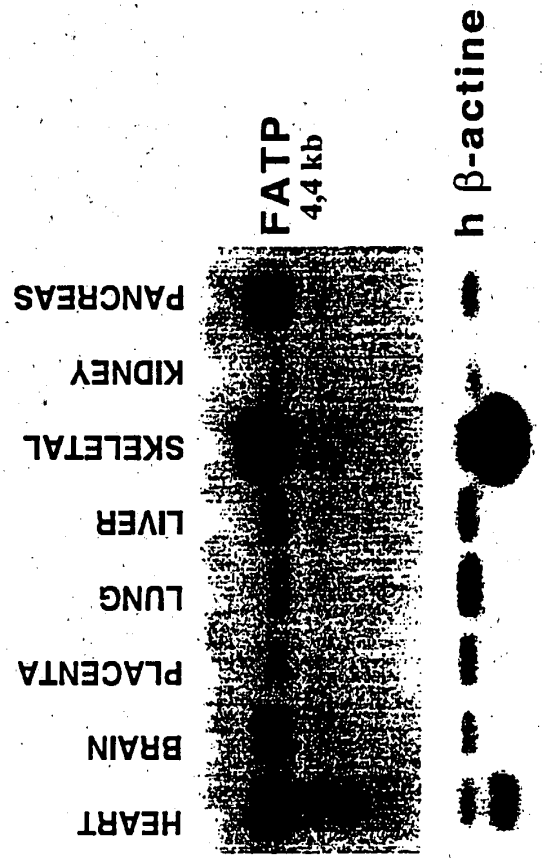

FATP
4,4 kb
h β-actine
HEART
BRAIN
PLACENTA
LUNG
LIVER
SKELETAL
KIDNEY
PANCREAS

FIG. - 17

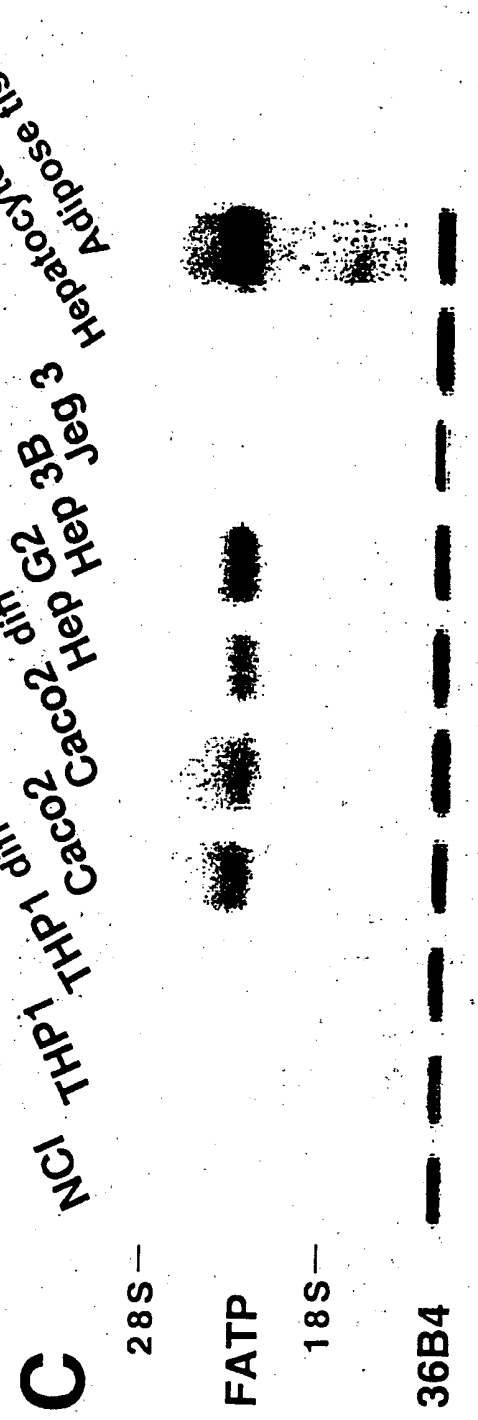

C
NCI
THP1
THP1 diff
Caco2
Caco2 diff
HepG2
Hep 3B
Jeg 3
Hepatocytes tissue
Adipose tissue
28S
FATP
18S
36B4

FIG. 18

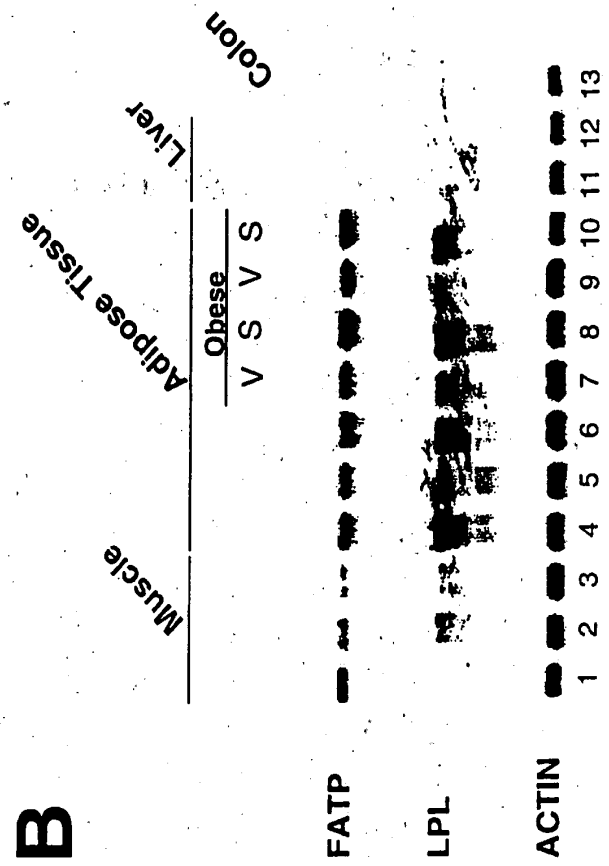

B
FATP
LPL
ACTIN
1 2 3 4 5 6 7 8 9 10 11 12 13
Obese
V S V S
Adipose Tissue
Liver
Colon
Muscle

FIG. 19